Determination of Telomere Length to Infer Forensic Age

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Abstract. Accurate age information is crucial for the identification of criminal cases in the field of forensics. Although the forensic age can be roughly estimated via bone age detection, there was no mature and reliable method for forensic age inference utilizing biological materials such as soft tissue fragments and no other markings left at the scene of the crime. Telomeres are cap-like structures found at the end of chromosomes in numerous tissues and cells and they are made up of repetitive sequences and binding proteins. This paper discussed the possibility of using telomere length to confirm the forensic age of a victim by concerning the biological characteristic of telomere and relevant techniques of length measuring. The structure of telomere enables it to prevent gene loss, fuse with other chromosome ends. Comparisons concentrating on the pros and cons of the existing measure of investigating the telomere are provided in this study by referring to those possible realistic scenes of the crime. As telomere length typically declines with every cell division as a result of the lagging strand's unsuccessful replication, the relationship between the shortening of telomere length and forensic are also shown for various types of cells. Further research in the field of investigating outliers in the pattern of shortening in telomere should be discovered and listed for various types of cells in order to make this method perfect and generalize it. This essay can provide some ideas for the study of telomere length inference of forensic age.

Keywords: Forensic medicine; telomere, telomere length; age estimation.

1. Introduction

Judging the forensic age is essential for forensic research and individual recognition. After confirming the real identity of the victim, by further investigation, the identity of the murderer and the cause of death of the victim will be revealed quickly, and it will be more conductive to the detection of the case.

The existing method for determinations the forensic age involves age estimation based on Deoxyribonucleic acid (DNA) methylation which refers to the changes of gene expression level based on non-gene sequence change and the degree of DNA methylation is negatively correlated with level of gene expression [1]. This assessment of age prediction is not suitable for small sample sizes, for example, using when the initial amount of the DNA sample is lower than 20 ng, the repeatability of the experiment will be challenged, and the difference between the results of repeated procedures can be higher than 5% [1]. The required fragment DNA is too long for amplifying those samples extracted from the scene several years ago. In cases where the skeleton meets the inference requirements, the biological age range of the source can be inferred by measuring the morphological changes of the bones and teeth with age, a typical example is by judging the degree of wear of the facies symphysialis. However, the main disadvantage of this measure is that the condition of the bone can be affected by many factors, which may lead to morphological variations and influence the age determination. Another introduced method is called forensic age estimation using Computed Tomography (CT) of the clavicle epiphyses [2]. The point needed to be concerned is that the clavicles epiphyses are not always in good condition when investigating the scene of the crime. The method of determination forensic age based on telomere length is a suitable, economic and convenient method referring to the identical feature of telomere DNA. This paper is a summary which includes most of the relevant information for further investigation and illustrates the necessity to promote this method as a constantly adapted way for investigating the forensic age.

This paper mainly focuses on the uses of telomere DNA to determine the forensic age. First of all, this paper introduced the importance, structure and function of telomere and the variations of telomere

between species briefly. After that, the variables determine the length are also listed and are categorized into several groups, two main groups are genetic and non-genetic factors, other worth mentioned special cases are also described in this topic. Furthermore, this study discussed the timeline of the techniques of measuring the telomere length and compared the pros and cons by referring to this purpose. At last, this paper mentioned the theoretical relationship between the telomere length and the age, including that of different kind of cells, such as T cells and corneal epithelial cells.

2. Telomere and Importance

The shelterin proteins and tandem DNA repeats known as telomeres guard the ends of chromosomes by forming T-loops [3]. Telomere length typically declines with every cell division as a result of the lagging strand's unsuccessful replication. Apoptosis or replicative senescence can result from prolonged telomere shortening or T-loop unwinding, which activates the DNA Damage Response [3]. Escaped cells are more likely to experience genetic instability, which raises the possibility of tumor development [4].

2.1. Length of Telomere in Different Species

Most creatures' telomere DNA is made up of relatively short, exact tandem repeating DNA sequences. The same telomere sequence can be found in animals that are extremely distantly related despite significant telomere DNA variation in many species. For instance, all vertebrates, Trypanosoma protozoa, and a number of gula bacteria and fungi share the same telomere sequence [5]. By species, telomere DNA average length varies. Humans weigh about l5 kb, while rats can weigh up to 150 kb, mice typically range from 5 to 80 kb, and caterpillars are barely 20 bp [5]. The telomere DNA length varies across all organisms. Depending on the genetic or nutritional state, yeast's telomere DNA can fluctuate by 200 to 400 ha [5]. During the logarithmic time scale, the telomere length of organisms like Tetrahymena and Trypanosoma rose steadily during the logarithmic phase [5].

2.2. Function

Telomere locates at the ends of chromatids of a DNA molecule. The main function of a telomere is to prevent gene loss during the process of DNA replication. It also prevents the telomeres from fusing each other. These functions depend on the characteristic structure of the telomere. Most of the repeating unit of a telomere have at least three guanines (G), so the single strand is called G-rich strand, forming the 3’ end of the telomere [6]. A G-quadruplex is formed when the conspicuous G-rich strand, which is longer than the complementary chain at the 3' end of the human telomere, is locked in the same plane by hydrogen bonding [7]. The lengthening of telomeres and DNA replication are both prevented by this structure. Chromosome ends are isolated by telomere loops (T-loops), which are formed by curling and looping the entire telomere. The displacement loop (D-loop), a three-stranded structure that is formed when the single-stranded DNA structure at the end of the telomere ring is incorporated into the double-stranded DNA of the telomere [8]. In order to maintain the integrity of chromosome structure and prevent the fusion of chromosome ends and ends, telomere structure is crucial. The function of telomere also relies on the combination between telomere and protein. Sheltering telomere binding protein is a complex of 6 proteins, involving TRF1, TRF2, RAP1, TIN2, TPP1 and POT1 [6]. The combination works by the inhibition of chromosome end DNA damage response mechanism and the control of the activity of telomerase on the chromosome end.

2.3. Factors Influencing Telomere DNA Length

Numerous variables that affect telomere length can be generally categorized as psychosocial, behavioral, environmental, tumor and disease-related, and other variables.

2.3.1 Formaldehyde

Formaldehyde is the main pollutant in China and the World Health Organization classifies it as a carcinogen [9]. It was found that it could influence the growth of cultured cells in vitro. High concentration of formaldehyde can inhibit the growth, while low concentration of that can promote the growth. The experiment involves twenty sexually mature Kunming mice (weight 22-32 g) were randomly divided into 1 blank control group and 3 infected groups, with 5 mice in each group [9]. Three formaldehyde solutions with different concentrations (1, 2 and 4 mg/mL, respectively) were prepared with 9% physiological saline [9]. The formaldehyde solution was fed once a day at 0.01 ml/g body weight (the dose was divided into 10, 20 and 40 mg/kg), and was fed continuously for 7 days. The control group was fed with 9% physiological saline [9]. Mice ate and drank freely. After the time period, the blood in the heart, liver and kidney tissue was extracted from each mouse through PCR (Polymerase Chain Reaction) method, the result is shown in the Table 1. As show in Table 1, the PCR products of the telomere of liver cell, kidney cell and blood cell all show that the length of the infected telomere is shorter than that of the control group.

Table 1. The relative length of telomere DNA sequence in different tissue cells of mice infected with formaldehyde (n=4, x±s, OD260)

Dosage(mg/kg)	blood cell	liver cell	kidney cell
0	0.944±0.024	1.147±0.008	0.807±0.116
10	0.914±0.020	0.894±0.047	0.814±0.091
20	0.832±0.024	0.782±0.080	0.713±0.008
40	0.682±0.037	0.673±0.028	0.711±0.064

2.3.2 Pesticides

Pregnancy-related pesticide exposure is linked to harmful health effects like low birth weight and poor neurodevelopment. Comparing women who lived close to agricultural fields to those who did not, it was found that the latter group had shorter telomeres ($p = 0.011$) [10]. Regular consumption of organic food was linked to shorter telomeres ($p = 0.01$), although eating other plants like artichokes was more likely to result in longer telomeres [10].

2.3.3 Genetic factor

Epidemiological studies have shown that telomere length is a complex genetic trait, with a heritability of 36% to 82% in twins and 34% to 50% in families [11]. A 2020 study comparing individuals of African ancestry with those of European ancestry showed that the difference in telomere length was mainly due to germ cells [12].

2.3.4 Non-genetic factor

Telomeres are rich in guanine and it can be easily oxidized. Under the condition of mild oxidative stress, the single chain will be preferentially accumulated on the telomere [11]. This can cause the replication fork stop functioning and incomplete replication at the end of a chromosome [13]. Exogenous factor should also be included. Studies have shown that smoking, becoming alcoholism, high-fat diet, obesity, sedentary, lack of exercise can cause oxidative stress and tissue inflammation which can accelerate the speed of telomere shortening. More detailed factors are summarized in table 2.

Table 2. Factors effecting telomere length

factor	conclusion	reference
Inheritance	the length of telomere can be passed to the next generation by maternal inheritance. There was a positive correlation between telomere length between older women and their offspring, but this is not shown for older men and their offspring.	[11]
Gender	Female adults have longer telomere than male adult, this is mainly because the hormone level difference. The negative correlation between the length of telomere length for a man and the age is greater than that foe women.	[14]
Stress level	The pressure is significantly related to the higher oxidative stress, lower telomerase activity and shorter telomere length of peripheral blood monocytes.	[15]
Disease	the shortening of telomere can promote the development of biological disease, appearance of cancer and further accelerates the shortening of telomere.	
Nutrient	Antioxidants and plant foods rich in antioxidants help maintain telomere length.	[16]
Age	According to an experiment, telomere length is negatively correlated with age. Telomere length was shorter in the aging population than younger objects of study, but no further reduction was shown in the telomere length after passing their 90s.	[17]

2.4. Effects of Telomere Length on Pregnant Women and Newborns

A total of 444 newborns were born in Gulou Hospital Affiliated to the School of Medicine of Nanjing University [18]. Questionnaire survey, physical examination and clinical indicators were performed on their mothers [18]. The umbilical vein blood and its matched maternal peripheral blood samples were collected. The relative telomere length of each sample was measured by quantitative PCR. Then paired T-test was used to compare maternal and infant telomere length, and general linear model was used to analyze the correlation between neonatal and maternal telomere length and other continuous variables. The telomere DNA in maternal cell was gradually lost through replication so the telomere length in the newborn was largely longer. The result shows that mother with longer years of schooling or more qualified mother has babies with longer telomere length [18]. Exposure to second-hand smoke, drinking and intaking tea during pregnancy have no significant impact on the relative telomere length of the mother and the newborn [18]. Drinking coffee during pregnancy can shorten the telomere length of the newborn [18].

3. Techniques of Measuring Telomere Length

In order to discover the telomere length, we have to select the most suitable method. Many measures with their own pros and cons have appeared and used in these days and I will explain each method and list their advantages and disadvantages referring to this experiment.

3.1. Southern Blot

Telomere was tested first in the 80s of the twentieth century. The first measure is called Southern blot (SB). The principle of the method can be summarized by using restriction enzyme to digest the sample DNA [19]. Through gel electrophoresis, fractions of DNA can be separated. Then transform it onto the nylon film can hybridize it with bio probe. The restriction of the method is that the result, telomere restriction fragment (TRF), can only show the telomere length of a cell but not the actual length of each telomere [19]. This measure will not be precise and accurate enough to illustrate the

correlation between the telomere length and the telomere because it may be uncertain. There may be slight difference between the telomere length inside a cell.

3.2. Q-FISH

In the 90s of the twentieth century, a breakthrough appeared as the telomere length would not be measured quantitatively. This method is time-consuming and asks for a large number of samples. Large amount of specimen cannot be tested at once means the method gives the user less chance for repeating the experiment. This can affect the quality of the result.

3.3. Flow-FISH

Based on the Q-FISH, this newly adapted method can be used to satisfy the demand for large-scale testing.

3.4. Q-PCR

By using upstream and downstream primer, enzyme, template and substrate, this method provides the user with an efficient measurement. One drawback of it is that it cannot provide the actual telomere length.

3.5. Surface Enhanced Raman Scattering (SERS)

The main principle of SERS method is that the nano-scale rough metal surface can enhance the Raman spectrum signal of adsorbent molecules. The simple operation was to select two single-stranded DNA as SERS probes, label two different Raman molecules, and hybridize with telomere and centromere specifically, respectively. The measurement result showed that the longer the telomere and the more fully hybridized with the probe, the stronger SERS signal was generated.

4. Relationship between Telomere DNA and Cell Division or Forensic Age

By investing the telomere length of people with ages ranging from 0-91, the result shows a negative correlation between the age and the length [5]. Approximately 50 bp will be shortened after each mitosis [20]. This phenomenon is believed to be common as it is shown in skin cells, epithelial cells, lymphocytes and stem cells etc.

Allsopp et al. observed the relationship between the initial telomere length and the numbers of mitosis of fibroblasts cultured in vitro from different individuals, and found that the longer the initial telomere length was, the more the number of mitosis and the stronger the ability to divide and proliferate [21].

4.1. TRF of Normal Gastric Mucosa Telomeres

An average of 41±12 bp of base pairs will lose annually. This discovery reveals that it can be used as a biological sign of aging.

4.2. Corneal Epithelial Cells and Restriction for Using Telomere Length to Predict Forensic Age

According to research, the TRF of corneal epithelial cell remains constant for samples with different age groups. Predicts for the possibility of the activation of the activity of telomerase was proved to be incorrect because the telomerase test was negative [5]. This specific condition might be related with the limited division ability for the human corneal epithelial cells.

4.3. T Cells and Relationship between Telomere Length and Ability for Replication

Being shorter than 7.0 kb shows that T cells begin to become senescent cells [5]. The telomeres of memory T cells were 1.4±0.1kb shorter than those of their precursor cells. The proliferation capacity of the latter was 128 times greater than that of the former, and the telomere length of both decreased

with cell division [5]. This indicates that telomere length is closely related to cell division and proliferation, and cells with long telomeres can carry out more cell division times. Telomeres are therefore considered markers of the cell's replication history and replication potential, and are the mitotic "clocks" that determine the cell's lifespan [5].

5. Conclusion

Telomere length is closely related to the forensic age. The dissertation focuses on the importance of telomere DNA by talking about the different length in different species and the special case for rapid length increase in logarithmic phase, the function of telomere which results from the structure of it, like the base sequence (TTAGGG), which rich in Guanine and the T loop isolates the telomere loops from the chromosome ends etc., genetic and non-genetic factors affecting the length of the telomere. It is believed that more advanced techniques will be invented and promoted to the forensic research of investigating the forensic age so that the forensic doctors can give the truth back to the case in a shorter period of time and maintain a more stable law-based society.

References

[1] Wang Z.W., Xu Q.N., Li C.T., Liu X.L., Age Estimation Based on DNA Methylation and Its Application Prospects in Forensic Medicine, Chinese Journal of Forensic Medicine, 2023, 01: 72-82.

[2] Wang D., Li P., Du Y.Z., Duan F., Zhang C.Y., Hao D.P., Forensic age estimation using CT of the clavicular epiphyses, Chinese Journal of Forensic Medicine, 2018, 04: 366-370.

[3] Evangelia S., Spyridoula G., Elisabeth R., Persefoni F., Aristides T., Dimitris T., Telomere and Telomerase, 2022, https://www.sciencedirect.com/science/article/abs/pii/B9780128243152000919.

[4] Lv J.Q., Shan S.F., Qiao T, Xu Y.J., Chen M.X., Cheng G., Xiong J.Y., Research progress on the correlation between telomere length and cancer, aging and dietary factors, Modern Preventive Medicine, 2020, 20: 3709-3712.

[5] Ge L.L., Liu C., Wang S.B., Research progress of telomere DNA and age correlation, Chinese Journal of Forensic Medicine, 2001, 02: 121-123.

[6] Yang J.Q., Huang Z.Y., Liang J., Xia Z.Y., Zhai X., Mo Y.N., Review of Telomere Length for Forensic Age Estimation, Journal of the Henan University of Science and Technology (Med Sci), 2016, 02: 156-160.

[7] Hansel R., Lohr F., Foldynova-Trantirkova S., et al., The parallel-quadruplex structure of vertebrate telomere repeat sequences is not the preferred folding topology under physiological conditions, Nucleic Acids Res, 2011, 39(13): 5768-5775.

[8] Zvereva M.I., Shcherbakova D.M., Dontsova O.A., Telomerase: structure,functions, and activity regulation, Biochemistry (Mosc), 2010, 75(13): 1563 − 1583 .

[9] Li H., Xiong D., Zhang Y.C., Ke Y.J., Qing X.D., Yang X., Ding S.M., Influence of formaldehyde on chromosome telomere DNA repeat sequence length in mice, Environ Health, 2013, 03: 202-204.

[10] Jaber H., Zia's A., Kifaya A., Tamar B., Kathryn J., Zohar B., Michael W., Influence of exposure to pesticides on telomere length and pregnancy outcome: Diethylphosphates but not Dimethylphosphates are associated with accelerated telomere attrition in a Palestinian cohort, 2023.

[11] Bai Y.J., Lin S..N, Wu D.Y., Regulation of telomere length and its relationship with a.ging and diseases, Chemistry of Life, 2022, 42(1): 101-110.

[12] Demanelis K., Jasmine F., Chen L.S., et al., Determinants of telomere length across human tissues, Science, 2020, 369(6509): eaaz6876.

[13] Victorelli S., Passos J.F., Telomeres and cell senescence-size matters not, EBioMedicine, 2017, 21: 14-20.

[14] Lulkiewicz M., Bajsert J., Kopczynski P., et al., Telomere length: how the length makes a difference, Mol Biol Rep, 2020, 47(9): 7181-7188.

[15] Turner K., Vasu V., Griffin D., Telomere biology and human phenotype, Cells, 2019, 8(1): 73-91.

[16] Freitas-Simoes T.M., Ros E., Sala-Vila A., Nutrients, foods, dietary patterns and telomere length: Update of epidemiological studies and randomized trials, Metabolism, 2016, 65(4): 406-415.

[17] Guo L.Y., Telomere length distribution and influencing factors in peripheral blood of healthy and long-lived individuals, master's thesis. 2021,

[18] Weng Q., Du J.B., Yu F., Jin G.F., Shen H.B., Hu Y.L., Influence factors for maternal and neonatal telomere length, Chinese Journal of Clinical Medicine, 2017, 06: 884-891.

[19] Ye X., Huang H.L., Progress of telomere length detection techniques and methods and comparison of their advantages and disadvantages, Medical Journal, 2018, 01: 148-150.

[20] Harley C.B., Futcher A.B., Greider C.W., Telomeres shorten during ageing of human fibroblasts, Nature, 1990, 345(6274): 458.

[21] Allsopp R.C., Vaziri H., Patterson C., et al., Telomere length predicts relative capacity of human fibroblasts, Proc Natl Acad Sci USA, 1992, 89(21): 10114.