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HETEROGENEITY IN TELOMERE LENGTH ON HUMAN CHROMOSOME

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ABSTRACT

Telomere sequence perceived at the end of each chromosome serve as an effective biomarker of a cell's replicative history. The average telomere length from blood and cheek cells of three persons of same age are found out by (terminal restriction fragment) TRF analysis. The concentration of isolated genomic DNA was determined and then double digested with restriction enzyme. By means of electrophoresis the length of telomere is calculated by comparing with DNA marker. Genetic variation in these telomeric region was also found out by DNA sequencing. The telomere from blood sample of third person showed a maximum length of more than10,000kb. Even though the blood sample and cheek cells from first and third person respectively showed same length, they differ in their genetic sequences. Various diseases like aging, cancer, anemia, Alzheimer, atherosclerosis, cardiovascular disease, hepatitis, chronic inflammatory bowel disease, and chronic HIV infection can be diagnosed by having a good knowledge on telomere length.

KEY WORDS

Biomarker, Cheek cells, DNA sequencing, Genetic variation, Telomere, Terminal restriction fragment.

INTRODUCTION

Each end of a chromosome is capped by a telomere, a nucleoprotein complex, which consists of non-coding TTAGGG double strand repeats, a 30 single strand overhang and associated telomere binding proteins [1]. Telomeres play an essential role in the maintenance of genomic stability because they act to protect the ends of chromosomes from DNA damage and prevent chromosomal end to end fusions [2]. Telomere content may serve as an effective biomarker of a cell's replicative history [3].Variations in the length of Telomere can induce replicative senescence, which blocks cell division. Alzheimer's disease (AD), a neurodegenerative disease is an example of premature ageing syndrome associated with telomere shortening.

Determining telomere length in case of abnormalities is a significant deed to eradicate complications. Telomere length can be assessed by a number of methods such as Telomere restriction fragment (TRF) analysis, quantitative PCR, single telomere length analysis (STELA), fluorescence *in situ* hybridization (FISH) and primed *in situ* (PRINS) labeling technique. Telomere length is measured from genomic DNA, cells and chromosomes. TRF analysis is the simplest and commonly used method. Here the genomic DNA is digested with specific restriction

enzyme, that doesn't cleavage telomeric sequences [4]. Subsequently gel electrophoresis and southern blots are carried out and the length of telomeres is revealed with a labeled telomeric probe.

The resultant DNA size distribution on the gel represents telomere lengths and the average length is calculated according to smear size and intensity. The size is assessed in kb so comparative studies can be made. TRF analysis is found to be very simple and easy. In this analysis, the genomic DNA is digested with restriction enzymes that do not recognize telomeric sequences. Subsequently electrophoresis is carried out to determine the length using DNA marker and genetic variation can be studied by sequencing. Telomere length abnormality is one of the earliest and most frequently acquired genetic alterations involved in the multistep process of malignant transformation. The present study is to determine the average length of telomeric DNA and compare it with individuals of relevant age. The size of telomeric DNA is also compared between different cell types of same individual this would bring a considerable contribution to human health care.

MATERIALS AND METHODS

Blood samples and cheek cells are collected from three persons at age 25. Individuals were selected from three different districts to study the effect of environmental factors on telomere length. DNA was extracted from blood and cheek cells by alkaline lysis method. The concentration and purity of DNA was determined by measuring the absorbance at 260nm using UV- Vis spectrophotometer and concentration was calculated using the formula

DNA (µg/ml) =OD_{260} x 50 x dilution factor

Agarose gel electrophoresis was performed to confirm the presence of genomic DNA. The extracted DNA was double digested with restriction endonuclease enzyme: *Hinf I* and *Rsa I.* The reaction was set up as given in **Table 1 and 2**.

The telomeric DNA separated from other DNA fragments by digestion can be visualized using 1.5% agarose gel electrophoresis. Along with the sample a DNA marker ranging to a size of 0.5-10kb was loaded in the well. Thus the size of telomeric DNA was determined by comparison with the marker. Telomeric DNA was extracted from agarose gel using MEDOX- Easy Spin Column DNA Gel Extraction Kit. The telomeric DNA sliced from the gel was combined with the binding buffer. Weight of each agarose gel slice (gel plus telomeric DNA) and the amount of binding buffer added is given in **Table 3, 4 and 5**.

Table 1: Quantity of reagents used for digestion of blood DNA

	Enzyme (10 U/µl)	Restriction enzyme buffer (µl)	DNA (µl)	Water(µl)
Person I	2.5	2	4.72	10.8
Person II	2.5	2	4.71	10.79
Person III	2.5	2	4.69	10.8

Table 2: Quantity of reagents used for digestion of DNA from cheek cells

	Enzyme (10U/µl)	Restriction enzyme buffer (µl)	DNA (µl)	Water (µl)
Person I	2.5	2	9.25	6.25
Person II	2.5	2	9.65	5.85
Person III	2.5	2	9.8	5.7

Table 3: Weight of gel slice and binding buffer added in sample of person I

Gel slice from	Weight (mg)	Binding buffer added (µl)
Blood	500	2000
Cheek cells	485.2	1940.8

Table 4: Weight of gel slice and binding buffer added in sample of person II

Gel slice from	Weight (mg)	Binding buffer added (µl)
Blood	491.1	1964.4
Cheek cells	489.0	1956

Table 5: Weight of gel slice and binding buffer added in sample of person III

Gel slice from	Weight (mg)	Binding buffer added (µl)
Blood	498	1992
Cheek cells	490	1960

Telomeric DNA extracted from the agarose gel was sent for sequencing to find out the genetic variation among individuals.

RESULTS

DNA was isolated from cheek cells and blood sample of three individuals. A linear band which represent intact DNA was obtained from all the samples (**Fig 1 a**). Concentration of DNA was determined by measuring the absorbance at 260nm with the help of UV- Vis spectrophotometer. In case of blood sample, the average concentration of DNA from person III was found to be more and that of cheek cells, person I has the maximum. The concentrations of each DNA sample are represented in following **Table 6 And 7**.

Table 6: Average concentration of DNA from blood sample

S.No	Concentration (µg/ml)
Person I	0.529±0.001
Person II	0.53 ±0.002
Person III	0.532± 0.002

Table 7: Average concentration of DNA from cheek cells

S.No	Concentration (µg/ml)
Person I	0.263±0.001
Person II	0.259 ±0.002
Person III	0.255 ±0.002

Table 8: Purity of each DNA sample from blood

S.No	Absorption A_{260}/A_{280nm}
Person I	1.83
Person II	1.79
Person III	1.8

Table 9: Purity of each DNA sample from cheek cells

S.No	Absorption A_{260}/A_{280nm}
Person I	1.8
Person II	1.82
Person III	1.88

Concentration of DNA isolated from blood was found to be more which shows that DNA isolated from blood sample was high. The purity of DNA was also calculated using **A_{260}:A_{280}** ratio the values are shown in **Tables 8 and 9**. The ratio between 1.8 and 2.0 gives high quality DNA. The value below 1.8 represent protein and value above 2.0 represent RNA. Protein was seen in blood sample of person II but its effect was negligible.

The telomeric DNA was isolated using electrophoresis. The non telomeric DNA fragments were seen near to the tracking dye and telomeric DNA separated from them. The length of each telomeric DNA was calculated by comparison with DNA marker. In case of person I, the average length of telomeric DNA (from blood sample) was found to be nearer to 10,000bp. But the telomeric DNA from cheek cells shows a decrease in length when compared with that of blood sample, which is above 8000bp (**Fig 1 b**). In person II, the length was found to range between 8000bp and 9000bp in case of blood sample and length was below 8000bp in case of cheek cells (**Fig 1 c**).In person III, the size of telomeric DNA (from blood sample) was greater than 10,000bp and that from cheek cells was near to 10,000bp (**Fig 1 d**).

The telomeric DNA separated from blood sample of person III showed maximum length. Even though the telomeric sequence extracted from blood and cheek cell of first and third person respectively showed similarity in length, they differ in their sequence. The sequences obtained from all three individuals were analyzed in BLAST (Basic local alignment search tool). The results are tabulated in **Tables 10, 11 and 12**.The telomeric DNA sequence from cheek sample of person I, blood sample of person II and cheek sample of person III showed 99% of maximum identity. Where the sequences of persons II(blood sample) and III(cheek sample) were referred with Homo sapiens DNA cross-link repair 1B (DCLRE1B), mRNA, Sequence ID: ref|NM_022836.3| and sequence of person I(cheek sample) with Homo sapiens platelet/endothelial cell adhesion molecule 1

(PECAM1), mRNA Sequence ID: ref|NM_000442.4|.

Table 10: BLAST results against Telomeric DNA sequence from cheek sample of person I Homo sapiens platelet/endothelial cell adhesion molecule 1 (PECAM1), mRNA Sequence ID: ref|NM_000442.4| Length: 6831Number of Matches: 1

Alignment statistics for match #1				
Score	**Expect**	**Identities**	**Gaps**	**Strand**
9274 bits(5022)	0.0	5035/5040(99%)	5/5040(0%)	Plus/Plus

Table 11: BLAST results against Telomeric DNA sequence from blood sample of person II against Homo sapiens DNA cross-link repair 1B (DCLRE1B), mRNA Sequence ID: ref|NM_022836.3| Length: 3795Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
6898 bits(3735)	0.0	3767/3780(99%)	12/3780(0%)	Plus/ Plus

Table 12: BLAST results against Telomeric DNA sequence from blood sample of person III against Homo sapiens DNA cross-link repair 1B (DCLRE1B), mRNA Sequence ID: ref|NM_022836.3| Length: 3795Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
6898 bits(3735)	0.0	3767/3780(99%)	12/3780(0%)	Plus/ Plus

DISCUSSION

The study aims in determining the average length of telomeric DNA and in contrast with individuals of same age. Among all the samples, the telomeric DNA separated from blood sample of the third person showed maximum length. Concentration of DNA was determined by measuring the absorbance at 260nm with the help of UV- Vis spectrophotometer. In case of blood sample, the average concentration of DNA from person III was found to be more and that of cheek cells, person I has the maximum. DNA was double digested with restriction endonuclease enzyme: *Hinf I* and *Rsa I.* These enzyme donot recognize DNA sequence present in the telomeric and subtelomeric regions of human chromosome. The *Rsa I* enzyme retained 100% activity in buffer for the *Hinf I* enzyme. These enzymes can cleave non- telomeric DNA to low molecular weight, so they move with electrophoresis gel front. The source *of Hinf I* enzyme is E.coli strain, that carries the cloned gene *Hinf I* from *Haemophilus influenza*. It is a type II restriction enzyme. It recognizes the double stranded sequence GANTC and cleaves after G-1, produces sticky ends. Catalytic activity of this enzyme is endonucleolytic cleavage of DNA to give specific double stranded fragments with terminal 5' phosphates. Optimal temperature of this enzyme is 37ºC but can be inactivated by heating to 65ºC for 15 minutes. The source *of Rsa I* enzyme is *Rhodopseudomonas sphaeroides*. It recognizes the double stranded sequence GTAC and cleaves

after T-1, producing blunt ends.The telomeric DNA was effectively eluted from the agarose gel and the samples were given for sequencing.

Sequencing would further precisely elicit the length and difference in the sequences among the three different persons. The size of telomeric DNA isolated from the blood sample of each person was greater when compared with that of cheek cells from same individual. The telomeric DNA separated from blood sample of person III showed maximum length. Eventhough the telomeric sequence extracted from blood and cheek cell of first and third person respectively showed similarity in length, they differ in their sequence. From this report, the accurate average length and sequence of each telomeric DNA was obtained. This variation depends on the ecological and environmental factors like obesity, smoking and psychological stress. The environmental factors may affect the length and cause a change in the base sequence of telomeric DNA even within the individuals of same age and among different cell types. Determination of average length of telomere suggests that various diseases like aging, cancer, alzheimer's etc can be diagnosed.

CONCLUSION

Selection of blood and cheek cells made the process of isolation of human genomic DNA simple and easy. Telomere length is an X-linked inheritance so DNA was extracted from three females of age 25. The genomic DNA was then double digested with restriction enzyme *Hinf I* and *Rsa I* that will not recognize telomeric and sub telomeric sequences. When the telomeric sequences were separated from agarose gel and compared with DNA marker, telomere from different samples showed different length. Thus it was summarized that average telomeric length can vary among cell types and between individuals. The telomeric DNA was then sequenced to study the genetic variation. From this report, the accurate average length and sequence of each telomeric DNA was obtained. This variation depends on the ecological and environmental factors like obesity, smoking and psychological stress. Telomere biology has tremendously enriched understanding of many cellular processes. During the last decades studies on telomere length have contributed only to carcinogenesis and age related disease, but now studies are related to diagnosis of various other diseases like atherosclerosis and associated cardiovascular disease, hepatitis, chronic inflammatory bowel disease, chronic HIV infection, different forms of anemia, and Alzheimer's disease. Telomere-driven genome instability that occurs during early stages of transformation process, represent the most widely spread cause of genome instability in cancer. Telomere length over the cell cycle has led to the consideration of telomeres as "mitotic clock". In response to aging, various cell intrinsic checkpoints and environmental factors will be altered that limit stem cell function. An understanding of the molecular pathways that induce these cell-intrinsic and cell-extrinsic alterations could ultimately lead to the development of new molecular therapies aiming to treat degenerative disorders and improve healthy ageing.

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