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Is telomere length one of the determinants of reproductive life span?

Received: 28 April 2004 / Accepted: 7 September 2004 / Published online: 3 May 2005
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Abstract The mechanism of final cessation of the reproductive life span has not been solved yet. It is generally assumed that the most important factor is ovarian follicular reserve. In ovaries at intrauterine period, a major factor that determines the number of the primordial follicle is the mitotic ability as well as the number of primordial germ cells, which migrate to gonadal ridge. The telomere length is one factor that determines the number of mitosis of the cell. The differences between the telomere lengths of same aged healthy women reflect the difference of the telomeres of the primordial germ cells at the intrauterine period. Women with long telomeres supposedly have had their primordial germ cells at the beginning of life with long telomeres. So, these cells should have had more mitotic division and more follicle numbers in the ovaries than the short ones. The aim of this study was to analyse the relation of the reproductive life span and telomere length. The telomere lengths of 37 women volunteers aged 50 years were measured by fiber FISH technique. A positive correlation was found between reproductive life span and the telomere length.

Keywords Fiber FISH · menopause · Reproductive life span · Telomere length

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Introduction

Menopause is the final cessation of menses, which is an irreversible end of a woman's reproductive life. Although the mechanism of menopause is still not well understood, it is generally assumed that menopause occurs as a result of depletion of the follicular reserve [6, 10, 14].

Although several environmental factors have been proposed as risk factors for the early onset of menopause [1, 3, 9, 13, 18, 22], factors influencing the timing of menopause are not well understood [17]. Some genetic factors have recently been proposed to be determinants of the age at which menopause occurs. This idea is strongly supported by the following studies; the twin study which showed the onset of menopause is genetically determined, yielding heritability for age at menopause of 63% [23]; the study on the association between mothers' and daughters' menopausal age [19]; and another study which showed family history as a predictor of early menopause [3].

Environmental and/or genetic factors, which are thought to have an effect on the timing of menopause, influence the rate of follicular reserve depletion at cellular level. Although the number of primordial germ cells and the rate of follicular atresia are supposed to effect the period of follicular reserve depletion, it is not clear if the affect of these factors together or separately act on follicular reserve depletion.

Factors that determine the number of primordial germ cells at the beginning are the number of the primordial germ cells that migrate to the gonadal ridge and the mitotic ability of these cells till gestational age 16–20 of the female fetus. Telomere length and telomerase activity can be two of the important factors that affect the mitotic ability.

Telomeres, the tandem repeats at the ends of the mammalian chromosomes, undergo attrition with each division of somatic cells in culture and their length is, hence, an indicator of replicative history and capacity of

these cells [2, 8, 11]. It can be assumed that the telomere length is effective as a mitotic clock, on the number of the intrauterine female primordial germ cells at the beginning. Therefore, the telomere length could also affect the reproductive life span.

It has been suggested that the length of telomeres in women of the same age reflects the length of telomeres at the beginning of life, and the difference between telomere lengths correlate with mitosis numbers of the primordial germ cells. For this reason, in the present study we aimed to show a possible relation between the telomere length and reproductive life span.

Materials and methods

Subjects

The subjects of this study were 37 healthy women all of them aged 50 years old, most of them were in menopause when measuring the length of the telomeres, whereas the others who were irregularly menstruating and reached menopause before we completed the study. After obtaining signed informed consent, 5 ml of peripheral blood was taken from every subject. Ankara University Ethical Committee approved the study (Approval # 34-793).

Reproductive story included; gestation number, live birth number, first delivery age, last delivery age, breast feeding duration and oral contraceptives usage. Ovulatory cycle indicators including menstruation regularity, frequency, duration, presence of dysmenorrhea, premenstrual tension, body mass index (BMI), smoking, economic and education status and meat consumption, which were all previously reported to have influence on the menopausal age, were recorded and evaluated together.

Fiber FISH

Dextrane sulphate (3.5%) was added to peripheral blood sample with EDTA with a final concentration of 12.3 $\mu\text{l/ml}$, and incubated for 1–2 h at 37°C. The upper phase was separated and then centrifuged at 2,000 rpm for 15 min. Fraction of white blood cells was suspended in PBS. DNA fibres were prepared using a previously described procedure [16, 20, 22] and FISHed using digoxigenin labeled all human telomeres probe (Oncor appligene CP5C97-DG) according to the previously described protocol [12, 21]. Rhodamine-labelled anti-digoxigenin antibodies (Oncor S1332-DR) were used for detection of probes, and fibres were counterstained with DAPI.

Slides were evaluated with Leica DM microscope using T \times 2 (for Rhodamine, excitation range is 560/40) and A (for DAPI, excitation range is 340–380) filters. For each material, lengths of minimally 50 probe signals (Fig. 1) were captured and measured with Q-FISH program.

Statistical analyses

Groups were compared by using Mann–Whitney *U*-test and Kruskal–Wallis variance analysis. The relation between reproductive life span and telomere length was tested using Spearman's correlation coefficient.

Results

Descriptive statistics of 37 healthy volunteers about menopause age, reproductive life span and telomere lengths are mentioned in Table 1.

The factors, which were previously reported to have an effect on menopause age and reproductive life span, were compared between groups by using Mann–Whitney *U*-test and Kruskal–Wallis variance analysis. It was found that there were no relations ($p > 0.05$).

The relation between reproductive life span and telomere length was tested using Spearman's correlation coefficient, and a positive correlation was found ($r = 0.673$, $p < 0.001$, Fig. 2).

Discussion

Follicular reserve is accepted as the most important factor for reproductive life span, though the mechanism of menopause has not been clarified yet [6, 10, 14, 15].

The factors that effect the duration of follicular reserve exhaustion are the number of the primordial germ cells migrated to gonadal ridge at intrauterine life, the mitosis number of the primordial germ cells at intrauterine period and follicular atresia rate at intra and extra uterine life [6]. It was reported that telomerase activity in ovaries was correlated with primordial follicle numbers and reproductive life span [7]. Since the replicative capacity of the primordial germ cells is affected by the telomere length of these cells that

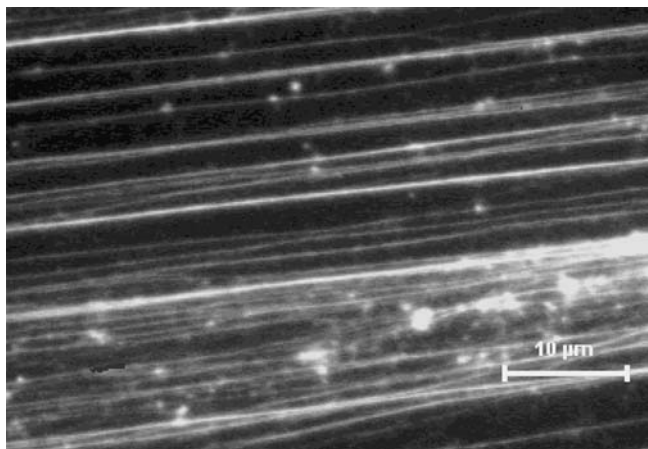


Fig. 1 Fiber FISH using digoxigenin labeled all human telomere probes [Oncor appligene CP5C97-DG]

Table 1 Descriptive statistics about menopause age, reproductive life span (RLS) and telomere lengths

	$X \pm SD$	Median (minimum-maximum)
Menopause age (years)	48.3 \pm 2.3	49 (43–51)
RLS (years)	35.6 \pm 2.6	36 (30–39.5)
Median telomere (μm)	3.4 \pm 0.5	3.2 (2.5–4.7)

migrated to gonadal ridge, it could be hypothesized that, apart from telomerase activity, telomere length of the primordial germ cells at the beginning of the intrauterine life of female fetuses is also important for the time of menopause of women. The replicative capacity of the primordial germ cells was affected by the telomere length of these cells that migrated to gonadal ridge.

Because the generally assumed menopause age is 50 years, we measured the telomere lengths of 50-year-old women. The women were either still menstruating or in menopause. The telomere lengths of these women who have different reproductive life spans and are assumed to have same telomere shortening, reflect the length of the telomeres of the primordial germ cells before mitotic division. Therefore, the difference between telomere lengths should have affected the mitosis numbers and so the follicle numbers. So, this difference determines the timing of menopause. Indeed, we found a significant correlation between the telomere lengths and reproductive life span, indicating the telomere length to be one of the factors affecting the reproductive life span.

Dorland et al. [5] studied general ageing, ovarian ageing and telomere length. In this study women older than 34 years of age, with unexplained infertility and had less than five oocytes after induced cycle were studied. The women's reproductive lives were accepted to cease soon. The researchers expected that if there was a relation between general ageing and ovarian aging, women who were accepted to have aged ovaries would have shorter telomere than the control group, fertile

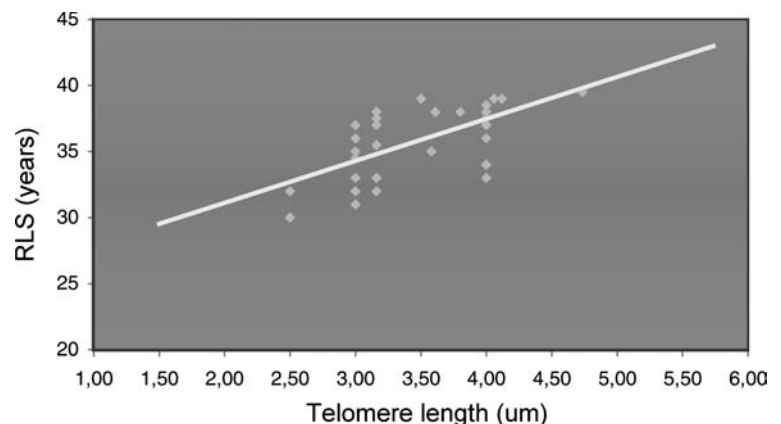
women. They were surprised because of the contrary results. These results showing that in the case infertile women, cell divisions in all cells were less than the control (fertile) group were interpreted as occurring probably due to growth hormone deficiency. This study also showed that there is a relation between reproductive life span and telomere length. The interpretation of the result of the study was that if the factor with negative effect on reproductivity and cell division was present, then cells' mitosis capacity would decrease. So, cells, in general, including leucocytes and primordial germ cells, divided less, and thus less primordial follicles were formed. For this reason, these women had less ovarian follicle, aged ovaries and also had long leucocyte telomeres.

In our study, the women had no problems of reproductivity. It could be supposed that the difference between telomere lengths of these women reflect the difference between division of primordial follicle numbers.

Evaluation of the results of these two researches together, in the normal range of reproductive life span determination, indicate that telomere length is an important factor, but the factors that affect cell cycle and division, which also affect mitosis number and reproductive life span, must always be kept in mind.

Up till now, there have been many studies on the effects of environmental and genetic factors on menopause age and reproductive life span. The mechanism of menopause and the factors that have an effect on it have not as yet been brought to light. To our knowledge, for the first time, a relation between telomere length and reproductive life span was investigated in this study. We found that telomere length effects reproductive life span considerably. The result needs to be clarified in large populations.

Acknowledgements This work was supported by Ankara University Research Foundation (Project No:2001-08-09-056). The authors are particularly grateful to Professor Isık Bökesoy and Professor Sevim Dinçer Cengiz for their support and valuable discussion.

Fig. 2 The correlation between reproductive life span (RLS) and telomere length

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