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Leukocyte telomere length and age at menopause

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Abstract

Background—Telomere length is a marker of cellular aging that varies by the individual, is inherited, and is highly correlated across somatic cell types within persons. Inter-individual telomere length variability may partly explain differences in reproductive aging rates. We examined whether leukocyte telomere length was associated with menopausal age.

Methods—We evaluated the relationship between leukocyte telomere length and age at natural menopause in 486 white women aged 65 years or older. We fit linear regression models adjusted for age, income, education, body mass index, physical activity, smoking, and alcohol intake. We repeated the analysis in women with surgical menopause. We also performed sensitivity analyses excluding women (1) with unilateral oophorectomy, (2) who were nulliparous, or (3) reporting menopausal age <40 years, among other exclusions.

Results—For every one kilobase (kb) increase in leukocyte telomere length, average age at natural menopause increased by 10.2 months (95% confidence interval= 1.3 to 19.0). There was no association in 179 women reporting surgical menopause. In all but one sensitivity analysis, the association between leukocyte telomere length and age at menopause became stronger. However, when excluding women with menopausal age <40 years, the association decreased to 7.5 months (−0.4 to 15.5).

Conclusions—Women with the longest leukocyte telomere length underwent menopause three years later than those with the shortest leukocyte telomere length. If artifactual, an association would likely also have been observed in women with surgical menopause. If these results are replicated, leukocyte telomere length may prove to be a useful predictor of age at menopause.

Telomere length is a marker of replicative aging in somatic cells and may also be a marker of reproductive aging. Telomeres are tandem repeats at the ends of chromosomes that shorten with each round of cell division in somatic cells. The reduction of telomere length to a critical threshold in cultured cells triggers replicative senescence[1]: the cell stops dividing and may also undergo apoptosis. The telomere can thus be viewed as a "mitotic clock," with its length indicative of the remaining replicative life of a cell. Shorter leukocyte telomere length has been associated with age-related diseases such as diabetes, insulin resistance,

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hypertension, atherosclerosis, and chronic heart failure.[2] Shorter leukocyte telomere length has also been associated with mortality.[3,4]

We propose that leukocyte telomere length may also be associated with female reproductive longevity. Infant girls are born with all the eggs they will ever produce. The eggs are arrested prenatally during meiosis; from the time of puberty, they gradually complete meiosis and are ovulated or undergo attrition. When few eggs remain, at an average age of 50–51 years in Western countries, reproductive senescence, or menopause, occurs. Menopausal age varies substantially, however, from 40 to 60 years,[5] and telomere length may be one of its determinants. Telomere length appears to be inherited[6–9] and is highly correlated across various tissues within an individual.[6,10–14] By influencing the number of possible cell divisions, telomere length could partly determine the number of primordial follicles and thus a woman's overall reproductive potential.

Studies examining telomere length and reproductive aging in humans, however, have yielded inconsistent results. Some studies have demonstrated an association between shorter leukocyte telomere length and earlier reproductive aging, including shorter reproductive lifespan and occult ovarian insufficiency[15–17] or poorer IVF outcomes,[18,19] while other studies have yielded negative or conflicting results.[20–22]

We therefore sought to investigate the relationship between leukocyte telomere length and reproductive aging, as expressed by age at menopause in a cohort of women age 65 years and older.

METHODS

Cardiovascular Health Study Cohort

The Cardiovascular Health Study is a multi-site longitudinal cohort study of 5,201 participants who were 65 years of age or older when recruited in 1989 and 1990 (the "original cohort") from Medicare eligibility lists in Forsyth County, North Carolina; Washington County, Maryland; Sacramento County, California; and Pittsburgh, Pennsylvania.[23] An additional 687 African-American participants were recruited into the study in 1992 and 1993 (the "African-American cohort"), for a total of 5,888 participants. From baseline until 1998 and 1999, participants completed up to 10 clinical visits in which data were obtained on vital signs, anthropometric factors, medical and reproductive history and behaviors, and physical and psychosocial functioning. Information on diet was also obtained for the original cohort at baseline. Participants were evaluated for cardiovascular outcomes at baseline and were extensively followed to collect data on incident cardiovascular disease, any hospitalizations, and mortality.[24]

Written informed consent was obtained at baseline and at specified intervals throughout the study. All procedures were in compliance with Institutional Review Board protocols for use of human subjects data.

Sample collection, processing, and storage

Each participant who consented to the use of their genetic material (~85%) was asked to provide a blood specimen from which DNA was extracted by using the PUREGENE® DNA Purification Kit (Gentra Systems; Minneapolis, MN).[25] DNA was then stored at −70°C before telomere length assessment.

Measuring telomere length

Eligible for this study were 948 women who completed the 1992–1993 clinic examination, consented to DNA use, and had leukocyte telomere length measurements. The integrity of the DNA was assessed through electrophoresis of 20 ng of DNA on one percent agarose gels (200 V for 2 hours) and staining with SYBR Green. Leukocyte telomere length was measured as the mean length of the terminal restriction fragments in peripheral leukocytes, using the Southern blot method as previously described.[26]

Each sample was analyzed on two occasions on different gels, and we used the mean of the two leukocyte telomere length values for all analyses. The Pearson correlation coefficient for the two measures within an individual was 0.97, with an average of 1.5% for the average coefficient of variation for pairs. The laboratory that quantifyied terminal restriction fragments was blinded to all subject characteristics.[27]

Age at menopause and other variables

Questions regarding women's reproductive characteristics, including age at menopause, history of hysterectomy or oophorectomy, and parity, were included in an interview completed at baseline. Self-reported demographic information (including race, education, marital status, and income) and medical and physical function variables were also collected at baseline. Body mass index (BMI) was measured by trained technicians during clinic examinations and was calculated as weight (kg) per height (m) squared. We used BMI and weekly alcohol intake information assessed at the visit corresponding to DNA collection for leukocyte telomere length measurement. For the original cohort, we also used information on cardiovascular disease assessed at year five.

Descriptive statistics

We first examined the number and percentage of participants within categories of various demographic, anthropometric, behavioral, and reproductive factors. We also examined the mean and standard deviation (SD) for leukocyte telomere length and age at menopause within categories of demographic, anthropometric, behavioral, reproductive, medical, and dietary variables.

Primary analysis

We used linear regression with robust standard errors to estimate the association between leukocyte telomere length and age at menopause and corresponding 95% confidence intervals (CI). Before beginning any data analyses, we constructed directed acyclic graphs[28] to identify factors expected to be confounders and therefore requiring adjustment in regression models: physical activity level before age 65 relative to peers (less active, same, more active), BMI (categorized as <25 kg/m^2, underweight/normal; 25–29.9, overweight; ≥30, obese), pack-years of smoking (continuous), weekly alcohol consumption (number of drinks, continuous), education (<high school; high school or equivalent; some college or vocational school; college or graduate degree), income (<$8,000; $8,000–15,999; $16,000–34,999; ≥$35,000), and age at the blood draw (continuous).

Women who reported a history of hysterectomy or bilateral oophorectomy prior to undergoing menopause (i.e. surgical menopause) were excluded – except for one sensitivity analysis to explore the association specifically in this group. Women with an age at menopause after age 60 or before age 30 years were also excluded, as these values were believed to be implausible and attributable to errors in recall. Due to concerns about the potential unreliability of self-reported age at menopause, we also excluded women with cognitive impairment or dementia [Mini-Mental State Examination (MMSE)[29] score ≥21]. The MMSE was administered in the original cohort but not in the African-American cohort,

who received a modified version (Modified Mini-Mental State Examination [3MSE])[30] and thus had scores on different scales. We restricted the primary analyses to white women because of this lack of correspondence, as well as for two other reasons: the relatively small number of non-white women meeting inclusion criteria in the original cohort, and the unexpected observation of strong effect modification (described below).

Additional confounders

Both leukocyte telomere length and age at menopause have been associated with dietary factors, including fiber,[31,32] polyunsaturated fats (particularly linoleic acid with telomere length),[31,33] and energy intake.[32,34] Because these findings are not conclusive and have not been replicated in all studies, we conducted an additional exploratory analysis in which we further adjusted the model for the daily average intake of dietary fiber (grams) over the whole year (continuous), the daily average intake of linoleic acid (grams) over the whole year (continuous), and the kilocalories consumed over the whole year (continuous).

Many factors are known or thought to be associated with menopausal age. Unless we were aware of a rationale for such factors' association with leukocyte telomere length, such as previously described associations or strong biological plausibility, we did not include them in the primary analysis. We did, however, perform secondary analyses that included as additional adjustment variables any factors we observed to be associated with both menopausal age and leukocyte telomere length (and presumably not in the causal pathway). Although confounding is best assessed by the magnitude of associations and not their p-values, there was no clear threshold that indicated a covariate was associated with leukocyte telomere length to a meaningful degree. Thus we considered as possible confounders those associated with both leukocyte telomere length (adjusted for age at blood draw) and menopausal age with $p<0.05$ in linear regression models.

Sensitivity analyses

In separate regressions, we re-fit the regression model restricted to women who underwent surgical menopause (n=179) or excluding women who (1) reported having undergone unilateral oophorectomy prior to menopause (n=19); (2) were nulliparous (n=10) or missing information regarding parity (n=72); (3) reported an age at menopause <40 years (n=26); (4) had a history of cardiovascular disease (CVD: myocardial infarction, stroke, atrial fibrillation, coronary heart disease, or congestive heart failure; n=101) or were missing information on CVD (n=3). Since age at surgical menopause should not be related to leukocyte telomere length, the first sensitivity analysis provides a crude check for bias or random findings. Prior studies suggest women do not accurately recall bilateral versus unilateral oophorectomy, and such misclassification of natural versus surgical menopause could dilute the study results. Nulliparity likely reflects subfertility in an unknown proportion of nulliparous women, yet the various reasons for subfertility could be postulated to correlate with both leukocyte telomere length and menopausal age either positively or negatively. A reported age at menopause of <40 years may not reflect natural menopause but could have been misreported or could have resulted from certain illnesses or treatment regimes (e.g., chemotherapy) that influence age at menopause. Lastly, CVD has been associated with shorter leukocyte telomere length and with age at menopause. Because it is unclear whether CVD influences age at menopause or whether menopausal status influences CVD risk, we performed a subanalysis in which we excluded women with CVD rather than adjusting for it.

Post-hoc analyses of effect modification

Although we did not anticipate that race would modify the association between leukocyte telomere length and age at menopause, we assessed this possibility in part to evaluate

whether the two CHS cohorts could be combined. We performed this assessment in the entire CHS cohort (both the original and African-American cohorts), without regard to cognitive impairment status. We also examined whether the leukocyte telomere length-age at menopause association was modified by age at the blood draw in white women regardless of cognitive impairment, as the rate of telomere attrition has been reported to change with age, and age at menopause appears to differ by birth cohort.[35]

RESULTS

Of the 948 women eligible for this study, 104 were from the African-American cohort; 38 were from the original cohort and non-white; 71 were missing age at menopause; 25 had a reported age at menopause <30 years (n=19) or >60 years (n=6); and 26 had moderate to severe cognitive impairment (Figure). Two hundred seventy participants had surgical menopause, and 42 were missing information on surgical menopause. An additional 45 participants were missing data on one or more confounders, resulting in 486 women for the primary analysis. The average age at menopause after excluding women with surgical menopause was 48.7 years (SD=5.3; range = 30–60) and the mean leukocyte telomere length was 6.3 kilobase pairs (kb) (SD=0.61; range = 4.8–8.4). The mean age at blood collection for leukocyte telomere length measurement was 74.9 years (range = 67–95). A majority of the women were married, had never smoked, and reported consuming no alcohol (Table 1). Characteristics were similar among the sample of white women from the original cohort with a leukocyte telomere length measurement, age at menopause, and no moderate or severe cognitive impairment (n=776) and the sample of women included in the primary analysis (n=486) (Table 1). The women who met at least one of the exclusion criterion were older, were less likely to be married, had lower levels of education, had lower annual incomes, and were more likely to report never smoking and consuming no alcohol (data not shown) than both the sample of white women without moderate or severe cognitive impairment and the primary analysis sample.

In age-adjusted linear regression analyses, we observed inverse relationships of leukocyte telomere length with parity; congestive heart failure; total kilocalories consumed in a year; daily average intake of fat, vitamin C, saturated fat, and cholesterol (eAppendix available online, www.epidem.com). Hypertension was positively related to leukocyte telomere length, and study site was also related to leukocyte telomere length. Pack-years of smoking, self-reported health status, and claudication were inversely associated with age at menopause, whereas education, income, and weekly alcohol consumption were positively associated. Parity was also related to age at menopause.

Leukocyte telomere length and age at menopause

Every one kb increase in leukocyte telomere length was associated with an average adjusted increase in age at natural menopause of 10.2 months (95% CI= 1.3 to 19.0) (Table 2). In women reporting surgical menopause, there was little to no association between leukocyte telomere length and age at menopause (−1.4 months [−18.7 to 15.8]). In sensitivity analyses excluding women who had undergone unilateral oophorectomy, were nulliparous, or had CVD, the association became slightly stronger (Table 2). However, when excluding women reporting an age at menopause of <40 years, the estimated association decreased to 7.5 months (−0.4 to 15.5).

Adjustment for dietary factors minimally strengthened the association (11.7 months [95% CI= 2.4 to 21.0]). Due to the observed and unanticipated association between leukocyte telomere length and parity (which is also associated with menopausal age), we assessed changes in estimated regression parameters excluding nulliparous women and adjusting for

multiparity (yes/no); the leukocyte telomere length-menopausal age association strengthened slightly (11.6 months; [1.0 to 21.2]).

In post-hoc analyses, we observed an interaction between race and leukocyte telomere length (p=0.03); each one-kb increase in leukocyte telomere length was associated with a 9.9-month increase in menopausal age in white women (95% CI= 1.2 to 18.6), but with a 17.3-month decrease in non-white women (−39.8 to 5.5). When we excluded the three most influential participants (all non-white) from this analysis, the effect modification weakened (p=0.18) but was qualitatively similar. The association between leukocyte telomere length and age at menopause was similar in women of all ages at blood draw (p=0.96).

DISCUSSION

We observed an average increase in age at menopause of 10 to 11 months per 1 kb increase in leukocyte telomere length. Differences in leukocyte telomere length, which ranged from 4.8 to 8.4, could thus potentially explain three-year differences in age at menopause. The results would be consistent with an even stronger association, given that errors in measuring leukocyte telomere length and self-reported menopausal age likely weakened the estimated magnitude compared with the true magnitude, if an association indeed exists. The association between leukocyte telomere length and reproductive lifespan as a whole may also be stronger since menopausal age is itself only a surrogate of this time period, the beginning of which occurs at menarche. Nonetheless, these results lend support to the notion that leukocyte telomere length may be a useful predictor of when a woman will be likely to reach the end of her reproductive lifespan.

We conducted several subanalyses to probe the robustness of the results to potential sources of bias. If an artifact, the association of menopausal age with leukocyte telomere length would likely also have been observed in women with surgical menopause, yet the estimated association in these 179 women was close to zero, with a very wide 95% CI. Subgroup analyses excluding nulliparous women or women with CVD strengthened the estimated relationship between menopausal age and leukocyte telomere length, suggesting that confounding by reproductive history or other health problems related to leukocyte telomere length does not account for the results. When we excluded women reporting an age at natural menopause of <40 years, the magnitude of the association decreased by 2.7 months; the 95% CI narrowed (despite the reduction in sample size) and covered a similar range. Thus the weakened estimate does not change the interpretation of the results. The difference in results by race was unanticipated and could be due to chance or unidentified selection bias. Evidence is accumulating that black women tend to have longer leukocyte telomere length than white women,[27,36] yet this difference alone cannot explain the inverse association with menopausal age. When we removed the three most influential observations the interaction was qualitatively similar but weakened greatly, suggesting that these women unduly influenced the strength of the estimated effect modification.

The plausibility of the study hypothesis rests on two assumptions: that leukocyte telomere length correlates with telomere lengths in ovarian granulosa cells and eggs, the decline of which causes menopause, and that postmenopausal telomere lengths reflect premenopausal telomere lengths. We are unaware of any studies investigating the correlation of telomere lengths between egg cells and leukocytes. However, telomere lengths exhibit synchrony across tissue types: even in the elderly, telomere length appears to be moderately or highly heritable,[6–9] and inter-individual variability is reportedly much greater than variability across cell types within individuals.[6,10–14] Thus, due to high intra-individual correlation across cell types and heritability over the lifespan, telomere length measured after

menopause and in leukocytes appears to be a useful marker of participants' pre-menopausal telomere lengths in egg cells.

A related assumption is that the rate of telomere attrition is similar pre- and post-menopause. This assumption could be faulty if, for example, the attrition rate accelerates after menopause, perhaps in response to a decrease in estrogen and its putative telomere-protective effects.[37] Such a difference could artificially exaggerate the results based on using post-menopausal telomere length as a surrogate for pre-menopausal length. This is because women with earlier menopause would experience a longer amount of time to undergo telomere attrition at a faster rate and thus might have shorter telomere length compared with women who had similar age-adjusted telomere length premenopausally but later age at menopause. Although it is plausible, we do not believe this source of bias accounts for the associations we observed, because we would then have expected to see a similarly biased relationship in the women with surgically induced menopause, i.e. a positive association between age at menopause and leukocyte telomere length. Yet, despite the similarity in the distributions of ages at surgically induced and natural menopause, the association with age at surgical menopause is slightly inverse and very close to null.

Furthermore, in an analysis of 1,156 subjects recruited in 4 longitudinal cohorts, after a mean of 12 years of follow-up 94% of subjects stayed within plus or minus one decile of their baseline leukocyte telomere length ranking, and approximately 50% remained in the same decile.[38] Almost half the subjects were women, with longitudinal time spans that covered pre- and post-menopausal periods; the results are consistent with a slow and stable rate of telomere length attrition over the adult life course.

Because we relied on self-reported covariates that were measured imprecisely, there is the possibility of residual confounding (i.e., incomplete adjustment for possible confounders). Adjustment for self-reported covariates decreased the association in our primary analysis by 2.1 months compared with the crude association. Use of more precise measures may have further attenuated the magnitude of the association; however, it is unlikely that it would reduce the association to the null. There is also the possibility of confounding by unmeasured covariates. The relative lack of data on determinants of leukocyte telomere length makes it difficult to specify other factors with plausibly strong associations as to cause confounding. We observed associations of leukocyte telomere length with age, race, and parity, but few other demographic or medical history characteristics. Adjustment for parity strengthened the results.

We are aware of only four previous reports regarding the relation of leukocyte telomere length specifically to age at menopause. Lack of such a relationship was reported in two large nested case-control studies of reproductive cancer using data from the Nurses' Health Study.[20,39] In both of these studies, as part of confounder assessment, associations were sought with relative leukocyte telomere length, measured using quantitative polymerase chain reaction (qPCR). In contrast, we assessed telomere length by using the Southern blot method, which provides a direct estimate of the average telomere length within a population of cells.[40,41] It is unknown whether a difference in the two methods of measuring leukocyte telomere length might explain the discrepancy. The Southern blot method is considered the gold standard,[42] and qPCR has a coefficient of variation nearly four times that of the Southern blot method,[41] which may partially explain the discrepant results. In one of the two studies (a breast cancer case-control study[39]), the inclusion of subjects having had previous hysterectomy and oophorectomy could also have biased the results toward the null. In two smaller studies, both of which relied on the qPCR assay method, leukocyte telomere length was positively correlated with age at menopause.[15,16]

Telomere length has more often been studied in women experiencing infertility, particularly those undergoing in vitro fertilization (IVF) or experiencing recurrent miscarriage. Longer average oocyte telomere length has been observed in women who become pregnant following IVF compared with those who fail to conceive,[43] and granulosa cell telomere length was shorter in women experiencing occult ovarian insufficiency compared with women experiencing male or tubal factor infertility.[17] Oocyte telomere length also appears to predict embryo fragmentation after IVF, which is a marker of embryo quality.[18] And 95 women with a history of recurrent miscarriage had shorter average telomere length than 108 women from the general population.[22] Yet these epidemiologic investigations have also not been entirely consistent, since longer average leukocyte telomere length was observed in women experiencing premature ovarian failure compared with women from the general population,[22] and poorly responding IVF patients older than 34 years with unexplained fertility disorder had longer lymphocyte telomere length compared with similarly aged fertile women.[21]

Previous authors have explained the seeming inconsistencies in findings by attributing greater relative importance to one of the multiple factors that affect the reproductive lifespan by influencing the number of primordial follicles: the number of primordial germ cells, their mitotic ability, and the activity of telomerase.[22] For example, it has been postulated that women with an unexplained fertility disorder may have a longer cell cycle, leading to fewer cell divisions and therefore fewer reductions in telomere length and a smaller pool of follicles.[21] We do not dispute the plausibility of such scenarios, in which the relationship between telomere length and menopausal age would depend on a woman's fertility status and reason for infertility. Nevertheless, their complexity underscores the need for prospective data collection in larger epidemiologic studies designed specifically to address the role of telomeres in determining reproductive potential. Such studies ideally would include reliable measurements of telomere length prior to occurrence of reproductive outcomes, as well as assessment of multiple facets of telomere biology. In addition, as the current analysis pertains to women who were of reproductive age primarily in the mid-1900s, collection of more recent data is warranted. Further investigations should also address some of the study limitations, such as the relatively small cohort resulting from the exclusion criteria and reliance on oral history of age at menopause collected many years after menopause.

The biological plausibility of the "telomere theory of reproductive senescence"[15,17,19] further argues for studies designed to validate the current results. Even in the absence of a known biological mechanism whereby telomere length directly influences age at menopause, due to synchrony leukocyte telomere length may still predict age at menopause or, more specifically, the probability of success of assisted reproductive technologies in specific patients. Thus if the observed association is replicated, leukocyte telomere length may prove to be a useful clinical biomarker of the end of the reproductive lifespan.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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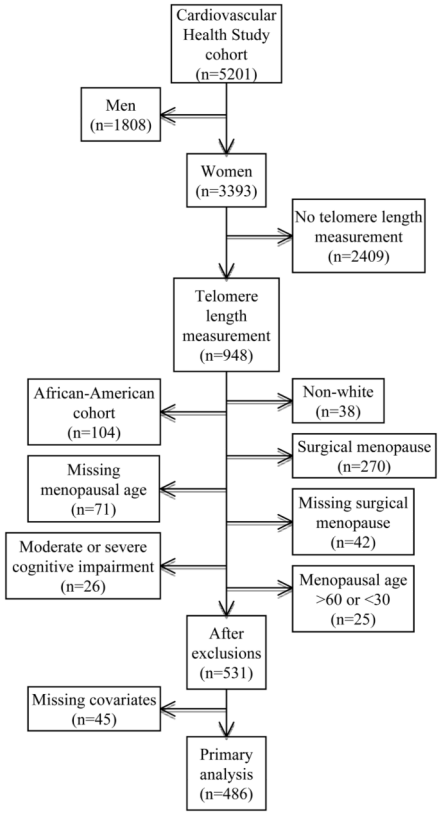

Figure.
Number of women excluded for various reasons in the primary analysis of the association between leukocyte telomere length and age at menopause in women older than 65 years who participated in the Cardiovascular Health Study.