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BRIEF REPORT

Shortened Telomere Length in Patients With Systemic Lupus Erythematosus

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Objective. **Patients with systemic lupus erythematosus (SLE) have a higher rate of premature death compared to the general population, suggesting a phenotype of premature senescence in SLE. Telomere length can be used to assess overall biologic aging. This study was undertaken to address the hypothesis that patients with SLE have reduced telomere length.**

Methods. **Telomere length was measured cross-sectionally in whole blood from SLE patients and age-matched healthy female controls, using real-time quantitative polymerase chain reaction. SLE-related and cardiovascular risk factors were assessed.**

Results. **We compared telomere length in 63 SLE patients and 63 matched controls with a median age of 50.8 years (interquartile range [IQR] 37–59 years) and 49.9 years (IQR 32–60 years), respectively. The median relative telomere length in SLE patients was 0.97 (IQR 0.47–1.57), compared to 1.53 (IQR 0.82–2.29) in controls ($P = 0.0008$). We then extended our cohort to measure telomere length in 164 SLE patients. Shorter telomere length was associated with Ro antibodies ($\beta \pm$ SE -0.36 ± 0.16; $P = 0.023$), and longer telomere length was associated with steroid therapy (0.29 ± 0.14; $P = 0.046$). We also noted an association of longer telomere length with increasing body mass index ($\beta \pm$ SE 0.07 ± 0.01; $P < 0.0001$) and tobacco smoking (0.64 ± 0.26; $P = 0.016$), as well as with the presence of carotid plaque (0.203 ± 0.177; $P = 0.032$).**

Conclusion. **Telomere length is shortened in SLE patients compared to controls and does not appear to be a reflection of disease activity or immune cell turnover. Subsets of patients such as those positive for Ro antibodies may be particularly susceptible to premature biologic aging. The predictive value of telomere length as a biomarker of future risk of damage/mortality in SLE requires longitudinal evaluation.**

Systemic lupus erythematosus (SLE) is associated with premature coronary heart disease and endothelial dysfunction (1). We have also noted a higher prevalence of subclinical atherosclerosis in SLE as assessed using high-resolution B-mode ultrasonography of the carotid wall (2). To date, the precise mechanisms underlying the early onset of atherosclerosis in SLE are unclear. However, the observation that atherosclerosis, a condition usually associated with aging, occurs at an earlier age in SLE patients leads to the hypothesis that premature biologic aging may occur in this population.

Telomeres are unique structures that form the physical ends of chromosomes and are believed to play a protective role. Telomeres consist of tandem repeats of 6 basepairs ending with a 150–200-nucleotide G-rich 3′ single-stranded overhang and associated proteins. Importantly, DNA polymerases are unable to replicate the lagging single strand of chromosomal ends, which results in telomere shortening with cell division; therefore, the

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length of telomeres can be considered to represent a cellular "biological clock" (3,4).

In this cross-sectional study, we measured whole blood telomere length in a cohort of SLE patients and age- and sex-matched healthy controls to address the hypothesis that patients with SLE have premature biologic senescence as indicated by shorter telomeres. We also investigated potential factors associated with telomere length in SLE.

PATIENTS AND METHODS

Patients were recruited from specialist SLE clinics across northwest England. All patients were women >18 years old; patients fulfilled the American College of Rheumatology revised criteria for SLE as updated in 1997 (5). Age-matched unaffected volunteers were recruited through an invitation from an SLE patient using a "buddy system," or through local advertisements. Participants were excluded if they were pregnant or lactating, or if they had a history of malignancy or had received treatment for malignancy in the preceding 12 months. All participants underwent a clinical assessment, which included assessment of disease activity using the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) (6) and damage using the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SDI) (7). Cardiovascular disease (CVD) risk factors were noted, and all patients underwent B-mode carotid ultrasound scanning to measure carotid intima-media thickness (IMT) and plaque as previously described (2).

Fasting blood samples were obtained from all participants to measure lipids and plasma glucose. In addition, a 5-ml sample of whole blood obtained at the time of the clinical assessment was frozen at −80°C until genomic DNA extraction was performed using an 8Lx automated DNA extractor. Average telomere length was measured by fluorescence-based real-time quantitative polymerase chain reaction (qPCR), using an adaptation of a previously described protocol (8), comparing a telomere repeat sequence copy number to a single-copy gene copy number in a given sample. All PCR analyses were undertaken with a LightCycler 480 real-time PCR instrument (Roche Diagnostics). All samples were measured in triplicate, and relative telomere length was calculated by determining the ratio of telomere length for each given sample to the telomere length of a randomly selected control sample to allow comparison between individuals. Measurement of a subset of 35 samples was repeated in triplicate at a second time point, and results demonstrated good correlation between all 6 measurements ($R^2 = 0.9$, $P < 0.0001$). Telomere length was compared between SLE patients and age-matched controls. To investigate contributing factors, telomere length was also measured in a larger cohort of SLE patients.

Data were analyzed using Stata 9.2 statistical software, and differences between SLE patients and healthy controls were assessed by 2-sample *t*-test for normally distributed continuous variables and by chi-square analysis for categorical variables. For non-normally distributed variables, nonparametric tests were used, i.e., the Kruskal-Wallis rank test for categorical variables and a standardized coefficient (β coefficient) in a regression model for continuous variables. *P* values less than 0.05 (2-sided) were considered significant.

RESULTS

We compared telomere length in 63 SLE patients (median age 50.8 years [interquartile range 37–59 years]) and 63 matched controls (median age 49.9 years [interquartile range 32–60 years]). The median relative telomere length in SLE patients was 0.97 (interquartile range 0.47–1.57) compared to 1.53 (interquartile range 0.82–2.29) in controls ($P = 0.0008$). We extended our cohort to measure telomere length in 164 SLE patients with a median age of 53 years (interquartile range 45–60 years). The median telomere length in this SLE group was 1.23 (interquartile range 0.64–1.86). Across the age range of our patients, age was not significantly correlated with telomere length.

We examined SLE-related factors associated with telomere length (Table 1). Overall disease activity (SLEDAI-2K) and damage (SDI) were not associated with telomere length. Higher C3, C4, and creatinine levels were associated with longer telomere length, and there was also a trend toward longer telomere length in patients receiving steroid therapy. Ro antibodies were associated with shorter telomere length, and there were negative trends associated with other autoantibody subtypes. With regard to traditional CVD risk factors in an age-adjusted analysis, several traditional risk factors actually had a positive association, including triglycerides, body mass index (BMI), and current smoking (Table 1). In particular, higher BMI was associated with longer telomere length ($R^2 = 0.25$). A backward stepwise multivariate model, using variables that were shown to have a significant relationship in the univariate analysis or could logically be confounders, was performed with a threshold for significance at $P < 0.2$. Associations with current steroid therapy ($\beta \pm$ SE 0.29 ± 0.14), Ro antibodies (-0.36 ± 0.16), BMI (0.07 ± 0.01), and current smoking status (0.64 ± 0.26) remained significant ($P = 0.046$, $P = 0.023$, $P < 0.0001$, and $P = 0.016$, respectively).

Telomere length was positively correlated with the presence of carotid plaque ($\beta \pm$ SE 0.203 ± 0.177; $P = 0.032$) but was not significantly associated with carotid IMT (-0.071 ± 0.797; *P* not significant). When telomere length was divided into 3 equal tertiles, a similar relationship with carotid IMT and plaque prev-

Table 1. Disease and CHD risk–related factors associated with telomere length in SLE (n = 164)*

SLE factors	Age-adjusted univariate model		Multivariate model†	
	$\beta \pm$ SE	*P*	$\beta \pm$ SE	*P*
Renal disease, yes/no	0.12773 ± 0.17973	0.33	–	–
Creatinine, per mole/liter	0.16633 ± 0.00194	0.04	0.01 ± 0.00	0.08
SDI, per unit	0.12404 ± 0.04656	0.11	−0.10 ± 0.06	0.07
SLEDAI, per unit	−0.05741 ± 0.01965	0.42	–	–
C3, per mg/ml	0.31485 ± 0.25900	<0.001	–	–
C4, per mg/ml	0.37374 ± 1.12487	<0.001	1.76 ± 1.08	0.11
dsDNA, per IU/ml	0.02564 ± 0.01986	0.82	–	–
Ro, positive/negative	−0.23895 ± 0.17638	0.002	−0.36 ± 0.16	0.023
La, positive/negative	−0.05462 ± 0.24992	0.34	–	–
Sm, positive/negative	−0.06435 ± 0.95228	0.36	–	–
RNP, positive/negative	−0.06367 ± 0.25736	0.27	–	–
Anticardiolipin antibody, positive/negative	−0.04967 ± 0.21726	0.32	–	–
Antimalarial use, yes/no	−0.00380 ± 0.18668	0.44	–	–
Immunosuppressant use, yes/no	0.12322 ± 0.18601	0.85	–	–
Azathioprine use, yes/no	0.00846 ± 0.16649	0.54	–	–
Steroid use current, yes/no	0.16036 ± 0.15875	0.05	0.29 ± 0.14	0.046
Disease duration, per year	−0.00595 ± 0.00830	0.94	–	–
HDL cholesterol, per mmole/liter	0.04469 ± 0.16177	0.44	–	–
LDL cholesterol, per mmole/liter	−0.02966 ± 0.08896	0.66	–	–
Triglycerides, per mmole/liter	0.12169 ± 0.12434	0.001	–	–
Systolic blood pressure, per 10 mm/Hg	0.08285 ± 0.00414	0.11	−0.01 ± 0.00	0.13
Fasting glucose, per mmole/liter	0.10656 ± 0.11144	0.24	–	–
Type 2 diabetes, yes/no	−0.02737 ± 0.33667	0.76	–	–
Family history of CHD, yes/no	−0.07698 ± 0.16511	0.33	–	–
Body mass index, per kg/m^2	0.50450 ± 0.01118	<0.001	0.07 ± 0.01	<0.0001
Current smoking, yes/no	0.16291 ± 0.24333	0.04	0.64 ± 0.26	0.016
Statin use, yes/no	0.03765 ± 0.16219	0.69	–	–
hsCRP, per mg/liter	0.13460 ± 0.01565	0.91	–	–

* CHD = coronary heart disease; SLE = systemic lupus erythematosus; SDI = Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index; SLEDAI = SLE Disease Activity Index; dsDNA = double-stranded DNA; HDL = high-density lipoprotein; LDL = low-density lipoprotein; hsCRP = high-sensitivity C-reactive protein.
† Performed for variables with a *P* value of less than 0.2 in the univariate model. R^2 for model = 0.57.

alence was observed (Table 2), and the prevalence of plaque was particularly associated with the highest tertile of telomere length.

DISCUSSION

We measured relative telomere length in peripheral blood mononuclear cells (PBMCs) from 63 patients with SLE, using qPCR. Consistent with our hypothesis, we demonstrated a significant reduction, of approximately one-third, of telomere length in SLE patients compared to age-matched controls. We also measured telomere length in a larger population of 164 SLE patients to assess factors associated with telomere shortening. Shorter telomere length was associated with Ro antibodies, and longer telomere length was associated with steroid therapy. Paradoxically, we also noted an association of longer telomere length with increasing BMI and smoking, as well as with the presence of carotid plaque. There was no significant correlation between age and telomere length in our study. However, it should be noted that the interquartile range of age in this patient group was 45–60 years, and previous studies have demonstrated that telomere length does not show a marked inverse correlation with age in this age range (9). We did not have access to a pediatric or adolescent SLE cohort; however, a study of younger patients with lupus would help to elucidate this relationship further.

Table 2. Measures of atherosclerosis associated with tertiles of telomeres in SLE (n = 164)*

Tertile of telomere length†	Marker of atherosclerosis	
	Plaque, %	Intima-media thickness, mean ± SD
Low	33	0.06 ± 0.02
Middle	38	0.06 ± 0.01
High	61	0.06 ± 0.01
P	0.02	NS

* SLE = systemic lupus erythematosus; NS = not significant.
† The low, middle, and high tertiles correspond to the shortest, intermediate, and longest telomere length, respectively.

Other studies have attempted to examine clinical associations with telomere length in SLE patients; however, these studies were limited by the data available and the sample size (with the largest study sample consisting of 60 patients) (10–12). Although previous studies have noted an association of telomere length with lymphocyte count, suggesting that telomere length may simply reflect lymphocyte turnover in SLE (10,11), we did not find such an association ($\beta \pm$ SE 0.01 ± 0.04; P not significant). Consistent with our findings, in a recent study, investigators using flow–fluorescence in situ hybridization to measure telomere length of PBMCs in 22 SLE patients and 20 controls (details of age not reported) did not detect a difference in telomere length in lymphocyte subpopulations between SLE patients and controls (12). Our study demonstrated that Ro antibodies were associated with telomere shortening. This may reflect disease subsets of SLE, such as subsets of patients with photosensitivity or Sjögren's syndrome spectrum overlap, who are most susceptible to premature aging.

We noted that there was a clear pattern of several factors associated with CVD risk, as well as the presence of carotid plaque, all positively associated with telomere length. These factors included BMI, triglycerides, and current smoking. This finding is in contrast to those of comparable small cross-sectional studies in non-lupus populations that have demonstrated an inverse correlation of BMI and tobacco smoking with telomere length (13). One possible explanation for the finding in our lupus cohort may be that these factors present low-level oxidative stress and a subsequent cellular response to lengthen telomeres. Alternatively, higher BMI may reflect better disease control with corticosteroid treatment. It should also be noted that a recent study of rheumatoid arthritis showed that higher BMI was actually associated with improved survival (14), and our study may reflect this in an SLE population.

Contradictory to our hypothesis, however, was our observation that carotid plaque was particularly prevalent in patients with the higher tertile of telomere length. The explanation for this is not clear; it may be that more of these patients are receiving statins and CVD-protective drugs that may increase telomere length. It may also reflect left censorship in our data. When considering factors associated with telomere length, a cross-sectional study such as this by definition studies only a "surviving cohort" from the whole population of SLE patients. As such, those patients with the shortest telomeres and atherosclerotic disease may have already died or have been too ill to take part in the study, and therefore, their data were not captured. This is supported by the fact that the highest tertile of telomere length was associated with plaque and it was not a graded association, suggesting a survival effect in this population. Longitudinal followup of this cohort will allow us to assess the influence of telomere length at a single time point on future CVD events and mortality. Interestingly, Cawthon et al have noted that even in older adults, shorter telomeres at a single time point do predict future adverse health outcomes (15).

We utilized real-time qPCR for this study, since this method yields rapid results and requires smaller amounts of DNA than Southern blotting. Previous studies have confirmed a good correlation between measurements with qPCR and Southern blotting (16). The primary outcome of our study is consistent with findings in previous smaller studies using Southern blotting that have demonstrated telomere shortening in SLE patients compared to controls. We studied an older population (median age 51 years) than previous studies (e.g., median age 34 years in Wu et al [11]) and confirmed this observation.

Our finding of shortened telomere length in SLE patients is interesting, and one possible explanation is that disease states lead to accelerated telomere attrition. Others believe an overall senescent phenotype will result in a propensity for certain diseases. Mesenchymal stem cells have recently been shown to have a senescent phenotype in a small study of SLE patients and may support the notion that premature senescence plays a role in the pathogenesis of SLE (17). The exact contribution of genetic factors on the length of telomeres also remains of much interest, and telomere maintenance genes have been shown to be altered in SLE patients with active disease (18). Further investigation is required to ascertain whether telomere length is reduced in individuals prior to the onset of autoimmune diseases as has been suggested by others in the context of rheumatoid arthritis (19). Serial measurements performed longitudinally rather than at a single time-point allowing the calculation of telomere attrition rate or quantification from an organ-specific cell type, may show a more definite correlation to clinical parameters.

Our study, therefore, supports the observations in smaller studies, which have shown a reduction in telomere length in SLE patients compared to controls (10,11). Similar to the results of previous studies, we did not find a correlation between telomere length and lymphocyte count or overall disease activity, suggesting that telomere length is not simply a reflection of immune cell turnover. We did, however, note that several subsets of patients, e.g., those with Ro antibodies or those with

lower C3 levels, had shorter telomere length, signifying that certain immunologic or clinical phenotypes may be associated with greater susceptibility to premature senescence. The main limitations of the current study are the narrow age range of the cohort and the cross-sectional design. Therefore, we are unable to determine if the associations we observed are the cause or the consequence of telomere length reduction. Longitudinal studies are essential to examine the clinical and prognostic significance of telomere shortening in patients with SLE.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Bruce had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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REFERENCES

1. Manzi S, Meilahn EN, Rairie JE, Conte CG, Medsger TA Jr, Jansen-McWilliams L, et al. Age-specific incidence rates of myocardial infarction and angina in women with systemic lupus erythematosus: comparison with the Framingham Study. Am J Epidemiol 1997;145:408–15.
2. Ahmad Y, Shelmerdine J, Bodill H, Lunt M, Pattrick MG, Teh LS, et al. Subclinical atherosclerosis in systemic lupus erythematosus (SLE): the relative contribution of classic risk factors and the lupus phenotype. Rheumatology (Oxford) 2007;46:983–8.
3. Chong L, van Steensel B, Broccoli D, Erdjument-Bromage H, Hanish J, Tempst P, et al. A human telomeric protein. Science 1995;270:1663–7.
4. De Lange T. Protection of mammalian telomeres. Oncogene 2002;21:532–40.
5. Hochberg MC, for the Diagnostic and Therapeutic Criteria Committee of the American College of Rheumatology. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus [letter]. Arthritis Rheum 1997;40:1725.
6. Gladman DD, Ibanez D, Urowitz MB. Systemic Lupus Erythematosus Disease Activity Index 2000. J Rheumatol 2002;29:288–91.
7. Gladman D, Ginzler E, Goldsmith C, Fortin P, Liang M, Urowitz M, et al. The development and initial validation of the Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index for systemic lupus erythematosus. Arthritis Rheum 1996;39:363–9.
8. Cawthon RM. Telomere length measurement by a novel monochrome multiplex quantitative PCR method. Nucleic Acids Res 2009;37:e21.
9. Aubert G, Lansdorp PM. Telomeres and aging. Physiol Rev 2008;88:557–79.
10. Honda M, Mengesha E, Albano S, Nichols WS, Wallace DJ, Metzger A, et al. Telomere shortening and decreased replicative potential, contrasted by continued proliferation of telomerase-positive CD8+CD28lo T cells in patients with systemic lupus erythematosus. Clin Immunol 2001;99:211–21.
11. Wu CH, Hsieh SC, Li KJ, Lu MC, Yu CL. Premature telomere shortening in polymorphonuclear neutrophils from patients with systemic lupus erythematosus is related to the lupus disease activity. Lupus 2007;16:265–72.
12. Beier F, Balabanov S, Amberger CC, Hartmann U, Manger K, Dietz K, et al. Telomere length analysis in monocytes and lymphocytes from patients with systemic lupus erythematosus using multi-color flow-FISH. Lupus 2007;16:955–62.
13. Valdes AM, Andrew T, Gardner JP, Kimura M, Oelsner E, Cherkas LF, et al. Obesity, cigarette smoking, and telomere length in women. Lancet 2005;366:662–4.
14. Wolfe F, Michaud K. Effect of body mass index on mortality and clinical status in rheumatoid arthritis. Arthritis Care Res (Hoboken) 2012;64:1471–9.
15. Cawthon RM, Smith KR, O'Brien E, Sivatchenko A, Kerber RA. Association between telomere length in blood and mortality in people aged 60 years or older. Lancet 2003;361:393–5.
16. Brouilette SW, Moore JS, McMahon AD, Thompson JR, Ford I, Shepherd J, et al. Telomere length, risk of coronary heart disease, and statin treatment in the West of Scotland Primary Prevention Study: a nested case-control study. Lancet 2007;369:107–14.
17. Nie Y, Lau C, Lie A, Chan G, Mok M. Defective phenotype of mesenchymal stem cells in patients with systemic lupus erythematosus. Lupus 2010;19:850–9.
18. Zhou JG, Qing YF, Yang QB, Xie WG, Zhao MC. Changes in the expression of telomere maintenance genes might play a role in the pathogenesis of systemic lupus erythematosus. Lupus 2011;20:820–8.
19. Steer SE, Williams FMK, Kato B, Gardner JP, Norman PJ, Hall MA, et al. Reduced telomere length in rheumatoid arthritis is independent of disease activity and duration. Ann Rheum Dis 2007;66:476–80.