A Prospective Study Investigating Prediagnostic Leukocyte Telomere Length and Risk of Developing Rheumatoid Arthritis in Women

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ABSTRACT. ***Objective.*** To prospectively examine the association between leukocyte telomere length (LTL) and subsequent rheumatoid arthritis (RA) development in women.

Methods. Using a case-control design nested within the prospective Nurses' Health Study (NHS), NHS II (NHSII), and Women's Health Study (WHS), each validated case of RA with a prediagnostic blood sample was matched to 3 controls by cohort, age, menopausal status, postmenopausal hormone therapy, and blood collection covariates. We measured telomere length in genomic DNA extracted from stored buffy coat samples using quantitative PCR. We used unconditional logistic regression to determine OR and 95% CI, and random-effects metaanalysis to combine study results.

Results. In total, we analyzed 296 incident RA cases and 827 matched controls. Mean age of diagnosis among women who developed RA was 60.5 in NHS/NHSII and 61.3 in WHS. Metaanalysis demonstrated that longer prediagnostic LTL was associated with increased RA risk when women in the longest versus shortest LTL tertile were compared (OR 1.51, 95% CI 1.03–2.23, $p_{heterogeneity} = 0.27$). However, statistically significant between-study heterogeneity was observed for the intermediate tertile category ($p_{heterogeneity} = 0.008$). We did not observe heterogeneity by menopausal status, inflammatory cytokine levels, age at diagnosis, age at blood collection, body mass index, seropositivity, or *HLA-DRβ1* shared epitope status.

Conclusion. Our results do not support an involvement for short LTL preceding RA development.
(J Rheumatol First Release January 15 2016; doi:10.3899/jrheum.150184)

Key Indexing Terms:
TELOMERE | RHEUMATOID ARTHRITIS | LEUKOCYTES
RISK ASSESSMENT | WOMEN | INCIDENT COHORT

Aging of the immune system, or immunosenescence, has been demonstrated to occur prematurely among individuals with rheumatoid arthritis (RA)[1,2,3]. Mechanisms are complex and not fully understood, but may involve changes in the adaptive immune system relevant to RA pathogenesis. With increasing age, a decrease in naive T cells occurs, most likely as a result of thymic involution, which is compensated by an increase in memory T cells. The shift in T cell homeostasis impairs T cell signaling by reducing T cell receptor diversity and proliferative capability, limiting antigen response. Similar changes occur in B cell populations with age[4]. Decline in T cell and B cell functions weakens the immune

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Supported by several grants from the US National Institutes of Health (P01 CA87969, R01 CA49449, UM1 CA176726, R01 CA67262, R01 HL043851, R01 CA047988, HL080467, HL099355, R01 AR049880, K24 AR066109, K24 AR052403, and R01 AR059073).

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Accepted for publication September 22, 2015.

system's ability to recognize "self" versus "foreign" antigens, and appears to promote a shift toward a proinflammatory cytokine profile, increasing the probability of autoimmune reactivity, infection, disease susceptibility, and mortality[3,5].

Patients with RA exhibit evidence of premature aging. Young patients with RA (age < 40) were found to have T cell telomere lengths, a marker of proliferative capacity[6,7], similar to participants > 65 years of age[8]. Extensive systemic inflammation during active RA, including elevated levels of tumor necrosis factor (TNF)-α, interferon-γ[9,10], and interleukin (IL) 6[11], may secondarily shorten telomeres. However, Koetz, *et al* did not find telomere length differences in patients by disease duration, activity, or severity[8]. Shorter telomeres and proliferation defects were noted in granulocytes[12] and hematopoietic progenitor cells[13] in patients compared with age-matched controls. Further, healthy individuals positive for *HLA-DRβ1* shared epitope status, a genetic risk factor for RA, displayed shorter telomeres than those who were negative, suggesting telomere defects may be intrinsic to RA rather than a consequence of increased proliferation or chronic inflammation[12].

Prospective epidemiologic studies of prediagnostic leukocyte telomere length (LTL) as a predictor of incident RA risk have not yet been performed, and could strengthen the argument for a fundamental involvement of telomere dysfunction in RA development. We used a nested, case-control approach among women from the prospective Nurses' Health Study (NHS), NHS II (NHSII), and Women's Health Study (WHS) to examine whether short prediagnostic LTL predisposes a woman to RA development. Additionally, we evaluated whether the relationship may be stronger among women with higher levels of inflammatory cytokines, more severe disease (seropositivity), or at genetic risk of RA (*HLA-DRβ1* shared epitope carriers).

MATERIALS AND METHODS

Study populations. The NHS was a prospective cohort following 121,700 female US-registered nurses aged 30–55 years in 1976. NHSII began in 1989 and enrolled 116,608 female US-registered nurses aged 25–42 years. At baseline and biennially thereafter, self-administered questionnaires gathered detailed information on lifestyle, reproductive factors, and medical history. Blood samples were collected in 1989–1990 from 32,826 NHS participants and in 1996–1999 from 29,611 NHSII participants. Upon arrival, blood samples were fractionated and stored in liquid nitrogen as plasma, erythrocytes, and buffy coat.

WHS was a completed, randomized, double-blinded, placebo-controlled trial of aspirin and vitamin E in the primary prevention of cancer and cardiovascular disease (CVD)[14,15]. Beginning in September 1992, 39,876 US female health professionals, predominantly white (> 94%), aged ≥ 45 years, and free of cancer or CVD, were enrolled in the study. Participants completed a detailed baseline questionnaire inquiring about their medical history and potential risk factors for cancer and CVD. Blood samples were collected from 28,345 (71%) women before randomization, which began in April 1993, and stored in liquid nitrogen freezers at –70°C.

We used a rigorous 2-step procedure, described in detail elsewhere[16,17], to validate self-reported, physician-diagnosed RA based on the 1987 American College of Rheumatology classification criteria[18]. Each cohort used a nested case-control design in which validated RA cases were included if they had donated a blood sample, were free of cancer (except nonmelanoma skin cancer), and developed RA after blood collection. Our analysis included 296 incident RA cases (149 in NHS, 73 in NHSII, 74 in WHS) with a blood sample collected at least 3 months prior to the onset of the first RA symptom documented in the medical record. Presence of rheumatoid factor (RF) and anticyclic citrullinated peptide (anti-CCP) positivity were extracted by medical record review. RF was available since 1976, while anti-CCP results were available starting in the early 1990s when the test became widely used. Seropositivity was defined as RF or anti-CCP positivity, and in NHS/NHSII supplemented by the anti-CCP assay in banked plasma samples[19].

For each validated case, we randomly selected 3 women who donated a blood sample, were free of cancer (except nonmelanoma skin cancer), and with no reported symptoms of connective tissue diseases up through the questionnaire cycle in which the case was diagnosed. We matched controls to cases by cohort, age (± 1 yr), blood collection characteristics, menopausal status, and recent postmenopausal hormone therapy (HT) use (< 3 vs ≥ 3 mos). Premenopausal women were also matched for timing of blood sample within the menstrual cycle. Additionally, WHS participants were matched for length of followup since randomization[16]. Because of racial differences in telomere dynamics[20,21,22], we restricted this analysis to self-reported white participants.

Completion of self-administered questionnaires and submission of the blood sample were considered to imply informed consent in the NHS and NHSII. Written informed consent was obtained from all WHS women before entry into the trial. All aspects of this project were approved by the Partners' HealthCare Institutional Review Board, Boston, Massachusetts, USA.

Laboratory assays. LTL in NHS/NHSII genomic DNA samples was assayed as previously described[23]. Triplicate reactions of each assay were performed on each sample. LTL is calculated as the exponentiated ratio of the telomere repeat copy number to a single-gene copy number (T/S) corrected for a reference DNA calibrator. NHS and NHSII samples were divided onto separate plates, but assayed as a single project. Telomere and single-gene assay coefficients of variation (CV) for triplicates were < 1%. LTL CV of blinded quality control (QC) samples interspersed within NHS and NHSII plates were 15.1% and 7.7%, respectively. To reduce misclassification, we calculated LTL for each sample replicate and excluded samples with within-replicate CV > 20% (n = 74, 8% of total).

Determining LTL. LTL in WHS genomic DNA samples was determined using a previously described quantitative PCR (qPCR)-based protocol[24], which differed from the qPCR method used by the NHS/NHSII. Telomere and single-copy gene amplifications were done in duplicate on the same 384-well plate. Cycle threshold value assignment was carried out by 2 independent observers, and if necessary, a complete reamplification was performed (< 3%). Duplicates of a no-template control and a reference DNA calibrator were included in each run for QC. Melting (dissociation) curve analysis was performed on every run to verify specificity and identity of the PCR products. T/S ratios were calculated blinded to case-control status.

Plasma assays. IL-6 was measured with an ultrasensitive quantitative sandwich ELISA (R&D Systems). We measured soluble TNF receptor II (sTNFRII) using a quantitative sandwich ELISA. The range of laboratory batch CV for each biomarker was 0.07–17.7% for IL-6 and 4.0–13.6% for sTNFRII. In NHS/NHSII, anti-CCP antibodies were tested through a second-generation, bead-based microarray using beads coated with peptide antigen (BioRad Laboratories). A fluorescence value of > 1500 was considered anti-CCP–positive because this cutoff was determined to have similar sensitivity/specificity to plate-based anti-CCP2 ELISA. Anti-CCP assays were not performed in the WHS because of limited sample availability. Laboratory personnel were blinded to case-control status of study samples. QC samples, indistinguishable from study specimens, were randomly interspersed throughout each plate. Detailed laboratory procedures and QC measures were previously reported[16].

HLA-DRβ1 genotyping of NHS/NHSII samples. Genomic DNA was whole-genome amplified with GE Healthcare Genomiphi (GE Healthcare Bio-Sciences Corp.). Low to intermediate resolution *HLA-DRβ1* typing was

performed on whole genome–amplified DNA using the LABType SSO (One Lambda) DNA typing method. Laboratory personnel were blinded to case-control status. Concordance for blinded samples was 100%.

Statistical analysis. Because the NHS and NHSII were assayed as a single project and we did not observe statistically significant between-study heterogeneity, data were pooled and referred to as a single "study," whereas WHS was a separate "study." Natural logarithm transformation of LTL, IL-6, and sTNFRII measurements improved normality prior to analysis. Using an extreme studentized deviate many-outlier procedure[25], we did not detect LTL outliers in either study. We used Spearman partial rank correlation coefficients to examine the relationship between LTL and potential confounders. Unconditional logistic regression models estimated OR and 95% CI for the association between LTL and incident RA risk. To evaluate whether risk differed by the seropositivity status of cases, we used polytomous logistic regression. Study-specific tertile categories of biomarkers were generated based on control distributions. All models were adjusted for matching factors. Adjusting for body mass index (BMI), pack-years of smoking, average alcohol intake, plasma IL-6, and sTNFRII levels did not change estimates by > 10% and thus were not included in final models. In the NHS/NHSII, we assessed paternal age at birth as a potential confounder because it is known to be related to LTL[26,27], as well as median family income and husband/partner's education level. Because estimates were not altered by > 10%, these factors were not included in final models. A per SD, LTL variable was modeled as continuous to calculate the Wald test for trend. Heterogeneity between studies was calculated using the Q statistic, and DerSimonian and Laird metaanalysis was used to combine study results[28]. We conducted stratified analyses by menopausal status (pre- vs postmenopausal at blood draw), time between blood draw and diagnosis (< 4, 4 to < 8, 8+ yrs), IL-6 levels (below vs above study-specific median), sTNFRII levels (below vs above study-specific median), median age at diagnosis (< 60, 60+ yrs), age at blood collection (≤ 50, > 50 yrs), BMI (normal vs overweight/obese), and *HLA-DRβ1* shared epitope status (0 vs 1 or 2 alleles), and tested interactions by the Wald test using cross-product terms. To assess heterogeneity by seropositivity, NHS/NHSII and WHS data were pooled, adjusted for study, and a likelihood ratio test performed. The Wilcoxon signed-rank sum test compared LTL by seropositivity and *HLA-DRβ1* shared epitope status. All p values are 2-sided with $\alpha = 0.05$. For our main hypothesis, we had 80% power to detect an OR of 1.68 comparing extreme tertiles using the formula of Chapman and Nam for ordinal exposures[29]. We used SAS Version 9.3 software (SAS Institute) for all analyses except polytomous logistic regression, which used STATA Version 12.1 software (StataCorp LP).

RESULTS

We present selected age-adjusted characteristics by study (NHS/NHSII, WHS) and disease status in Table 1. Prediagnostic blood samples collected from NHS/NHSII cases occurred at a slightly younger age (52.0 yrs) than for WHS cases (55.4 yrs). Because of a longer interval between blood collection and RA diagnosis for NHS/NHSII cases (8.5 yrs) compared with WHS cases (5.8 yrs), the mean age at diagnosis was similar between studies (60.5 vs 61.3 yrs). Cases and controls were of similar age and menopausal/HT use status within each study at blood collection. WHS women were slightly older, more likely postmenopausal and to have used HT, but less likely to be current smokers than NHS/NHSII participants. Cases within each study had higher BMI than controls. Pre-RA cases from NHS/NHSII consumed less alcohol than matched controls, whereas WHS women consumed similar amounts of alcohol regardless of case status. Plasma levels of sTNFRII, a proxy for the rapidly degraded inflammatory cytokine TNFα, and IL-6 were slightly higher in pre-RA cases compared with controls in both studies. LTL between studies were not directly comparable because of different qPCR protocols and calibrator DNA.

We examined correlations between LTL, age at blood collection, BMI, pack-years of smoking, alcohol intake, IL-6, sTNFRII, husband/partner's education (NHS/NHSII only), and median family income (NHS/NHSII only) among controls. While the correlation between LTL and age was in the expected direction in both studies (NHS/NHSII: $r_s = -0.05$, $p = 0.22$; WHS: $r_s = -0.06$, $p = 0.38$), the associations were not significant. No other characteristic was significantly related to LTL in either study (data not shown).

Women in the longest versus shortest LTL tertile had an OR 1.75 of developing incident RA (95% CI 1.16–2.62, $p_{trend} = 0.03$; Table 2) in NHS/NHSII, but not in WHS (OR 1.15, 95% CI 0.62–2.13). With the exception of the intermediate LTL category, we did not observe significant heterogeneity between studies. Using random-effects metaanalysis, prediagnostic LTL increased RA risk comparing the longest versus shortest LTL tertile (OR 1.51, 95% CI 1.03–2.23, $p_{heterogeneity} = 0.27$), and the per-SD estimate (OR 1.18, 95% CI 1.03–1.35, $p_{trend} = 0.02$, $p_{heterogeneity} = 0.85$). Estimates remained the same when NHS/NHSII and WHS data were pooled. In sensitivity analyses, the pattern of association was similar when LTL was dichotomized at the median value, categorized as quartiles, or modeled continuously (data not shown).

Because RA risk factors may differ for premenopausal compared with postmenopausal women[30,31], we conducted analyses stratified by menopausal status. RA risk associated with LTL did not differ significantly by menopausal status at blood draw in our study ($p_{heterogeneity} = 0.70$). For those with longest versus shortest LTL, the OR was 1.73 (95% CI 0.94–3.17) among premenopausal and 1.45 (95% CI 0.90–2.32; Table 3) among postmenopausal women. We also performed stratified analyses by length of time between blood collection and RA diagnosis to investigate whether preclinical disease influenced results. Evidence for increased RA risk associated with the longest LTL tertile was observed among women who donated a blood sample at least 8 years prior to diagnosis (OR 1.78, 95% CI 1.08–2.95, $p_{trend} = 0.05$, $p_{heterogeneity}$ by time since blood draw = 0.85; Table 4). Risk of RA associated with LTL did not differ between women with high versus low plasma IL-6 ($p_{heterogeneity} = 0.74$) or sTNFRII levels ($p_{heterogeneity} = 0.94$), by median age at diagnosis ($p_{heterogeneity} = 0.34$), age at blood collection ($p_{heterogeneity} = 0.76$), or BMI ($p_{heterogeneity} = 0.92$; data not shown).

Prior studies observed significant differences in LTL by RF positivity[8] or *HLA-DRβ1* shared epitope carrier status[12]. In our study, LTL did not significantly differ by serostatus among NHS/NHSII ($p = 0.76$) or WHS pre-RA cases ($p = 0.08$). Metaanalysis of polytomous logistic regression results revealed somewhat stronger risk for seropositive RA (OR 1.74, 95% CI 1.15–2.64, $p_{trend} = 0.01$) than seronegative RA

Table 1. Age-standardized characteristics of participants prior to blood draw by study. Values are mean (SD) unless otherwise specified.

Characteristic	Nurses' Health Studies		Women's Health Study	
	Cases, n= 222	Controls, n = 584	Cases, n = 74	Controls, n = 243
Age at blood draw, yrs*	52.0 (7.8)	51.6 (8.0)	55.4 (7.7)	55.4 (7.5)
Age at RA diagnosis, yrs*	60.5 (9.9)	NR	61.3 (7.7)	NR
Time to RA onset, yrs*	8.5 (4.9)	NR	5.8 (2.5)	NR
Seropositive, RF and/or anti-CCP, %	59	NR	58	NR
Radiographic changes**	23	NR	23	NR
Smoking status, %				
Never	44	49	35	49
Past	42	36	57	42
Current	15	14	8	8
Body mass index, kg/m^2	26.1 (5.1)	25.0 (4.3)	26.1 (5.9)	25.7 (5.0)
Average alcohol intake, g/day	4.4 (6.2)	5.6 (8.3)	5.7 (10.9)	5.4 (10.2)
Husband/partner's education %				
< 4 yrs of college	36	33		
4-yr college	26	27		
Graduate school	22	25		
Missing/not applicable	16	15		
Median family income, %				
< $40,000	12	11		
$40,000–$49,999	20	20		
$50,000–$64,999	31	31		
$65,000–$79,999	20	19		
$80,000+	18	19		
Menopausal status, %				
Premenopausal	36	36	24	26
Postmenopausal, no HT use	23	25	16	13
Postmenopausal, HT use	31	29	40	40
Menopausal status unknown	11	11	20	22
IL-6, pg/ml	1.6 (1.7)	1.4 (1.4)	2.3 (2.8)	2.1 (2.5)
sTNFRII, pg/ml	2427 (716)	2406 (757)	2320 (666)	2200 (585)
Median LTL†	0.62	0.59	0.24	0.21

* Value is not age-adjusted. ** Erosions or periarticular osteopenia consistent with RA. † Different protocol and calibrator DNA used in the Nurses' Health Studies versus the Women's Health Study. RA: rheumatoid arthritis; RF: rheumatoid factor; anti-CCP: anticyclic citrullinated peptide antibodies; HT: hormone therapy; IL-6: interleukin 6; sTNFRII: soluble tumor necrosis factor receptor II; LTL: leukocyte telomere length; NR: not relevant.

Table 2. Association of LTL with incident RA risk by cohort*.

Variables	Nurses' Health Studies			Women's Health Study			Metaanalysis		p_{het}
	Ca/Co, n	OR	95% CI	Ca/Co, n	OR	95% CI	OR	95% CI	
Shortest tertile	51/195	1.00		25/76	1.00		1.00		
Intermediate	85/191	1.76	1.17–2.64	17/84	0.59	0.29–1.19	1.06	0.36–3.09	0.008
Longest tertile	86/198	1.75	1.16–2.62	32/83	1.15	0.62–2.13	1.51	1.03–2.23	0.27
Per SD**		1.19	1.01–1.40		1.15	0.88–1.51	1.18	1.03–1.35	0.85
p_{trend}†		0.03			0.30		0.02		

* Unconditional logistic regression adjusted for matching factors: age (continuous), menopausal status/HT use at blood draw (premenopausal, postmenopausal-no HT, postmenopausal-current HT, unknown), and cohort (only for NHS, NHSII). ** Nurses' Health Study LTL SD = 0.26; Women's Health Study LTL SD = 0.71. † Based on per SD analysis. LTL: leukocyte telomere length; RA: rheumatoid arthritis; Ca: cases; Co: controls; p_{het}: $p_{heterogeneity}$; HT: hormone therapy; NHS: Nurses' Health Studies; NHSII: NHS II.

(OR 1.15, 95% CI 0.48–2.76, p_{trend} = 0.76) associated with the longest LTL tertile, but the test for heterogeneity was not significant (p = 0.37; Supplementary Table 1, available online at jrheum.org). Risk associated with the longest LTL tertile did not differ by *HLA-DRβ1* shared epitope carrier status (p = 0.45; Supplementary Table 2, available online at jrheum.org). LTL did not differ by carrier status in either the entire population (p = 0.62), case-only (p = 0.66), or control-only (p = 0.59) groups.

DISCUSSION

Using a nested case-control design in 3 large prospective cohorts of women, our study includes the largest number of cases to date, and is the first to investigate LTL prior to onset

Table 3. Association of LTL with incident RA risk by menopausal status in the Nurses' Health Studies and the Women's Health Study combined*.

Variables	Premenopause			Postmenopause			p_{het}
	Ca/Co, n	OR	95% CI	Ca/Co, n	OR	95% CI	
Shortest tertile	26/95	1.00		39/135	1.00		
Intermediate	35/96	1.40	0.78–2.52	58/150	0.98‡	0.24–4.01	
Longest tertile	35/81	1.73	0.94–3.17	64/155	1.45	0.90–2.32	
Per SD**		1.13	0.89–1.43		1.18	0.97–1.42	
p_{trend}†		0.31			0.10		0.70

* Metaanalysis used to combine estimates from unconditional logistic regression analyses adjusted for the same covariates listed in Table 2. ** Nurses' Health Study LTL SD = 0.26; Women's Health Study LTL SD = 0.71. † Based on per SD analysis. ‡ Significant heterogeneity between studies (p = 0.01). LTL: leukocyte telomere length; RA: rheumatoid arthritis; Ca: cases; Co: controls; p_{het}: $p_{heterogeneity}$.

Table 4. Association of LTL with incident RA risk by time since blood draw to diagnosis in the Nurses' Health Studies and the Women's Health Study combined*.

Variables	< 4 Yrs			4 to < 8 Yrs			8+ Yrs			p_{het}
	Ca/Co, n	OR	95% CI	Ca/Co, n	OR	95% CI	Ca/Co, n	OR	95% CI	
Shortest tertile	13/52	1.00		28/83	1.00		31/115	1.00		
Intermediate	26/71	1.13	0.18–7.16	31/89	1.06	0.32–3.51	41/92	1.58	0.94–2.67	
Longest tertile	34/80	1.78	0.83–3.80	29/78	1.21	0.64–2.29	51/105	1.78	1.08–2.95	
Per SD**		1.20	0.91–1.59		1.14	0.87–1.48		1.22	1.00–1.49	
p_{trend}†		0.19			0.35			0.05		0.85

* Metaanalysis used to combine estimates from unconditional logistic regression adjusted for the same covariates listed in Table 2. ** Nurses' Health Study LTL SD = 0.26; Women's Health Study LTL SD = 0.71. † Based on per SD analysis. LTL: leukocyte telomere length; RA: rheumatoid arthritis; Ca: cases; Co: controls; p_{het}: $p_{heterogeneity}$.

of RA symptoms. Our results do not support short LTL as an RA risk factor. Instead, we observed potentially increased RA risk among women with long LTL. Women in the longest versus shortest LTL tertile had a summary OR 1.51 (95% CI 1.03–2.23), which did not significantly differ by menopausal status, inflammatory biomarker levels, age at blood collection, age at RA diagnosis, BMI, serostatus, or genetic risk of RA. Because many trends did not reach significance, our observations could be due to chance.

Our results are inconsistent with the literature on the relation between LTL and RA risk, possibly because of the differences in study design and population (Supplementary Table 3, available online at jrheum.org). Using prospective cohorts, we investigated whether telomere length differed among women who later developed RA from those who did not. Prior studies used cross-sectional designs[8,12,13,32], in which established and treated patients with RA exhibited shorter telomere lengths across hematopoietic cell lineages compared with controls[8,12,13,32]. Some studies suggested shorter telomeres were restricted to young patients with RA (age < 40), with telomere lengths indistinguishable between cases and controls at older ages[8,12,13]. However, a larger independent study observed shorter LTL among RA cases in every age group[32]. In our study, RA risk associated with the longest LTL tertile was not reduced, but rather elevated regardless of age or menopausal status. However, most of our cases were ≥ 40 years of age (97%) at the time of blood collection. While our observations could be due to chance, a similar discrepancy was observed for non-Hodgkin lymphoma (NHL) risk by study design. A retrospective study of newly diagnosed aggressive NHL cases observed significantly higher risk associated with short telomeres[33], whereas 2 independent prospective studies observed significantly increased NHL risk associated with longer prediagnostic LTL[34,35].

RA cases from academic medical centers may represent the more severe end of the disease spectrum compared with patients managed in the community[32]. Our case population, from cohorts of women in the United States, may identify a broader distribution of RA severity compared with prior studies. Patients with RA with greater disease severity are more likely RF-positive[36] and carriers of *HLA-DRβ1* shared epitope alleles[37,38]. Two prior studies included a higher proportion (84% and 100%) of RF-positive patients[8,13] than the proportion of seropositive patients in our study (58% and 59%). However, we observed that the longest, rather than the shortest, LTL tertile was associated with increased seropositive RA risk. LTL did not differ by seropositivity among these pre-RA cases, consistent with 1 prior study[32], but not another[8] among patients with established RA.

We did not observe significant heterogeneity in the association between LTL and RA risk by *HLA-DRβ1* shared epitope carrier status. Elevated risk was associated with the longest versus shortest LTL tertile in carriers and noncarriers, and

LTL did not differ by carrier status in our study. In Steer, *et al*[32], patients with RA who were carriers had shorter LTL than noncarriers, but this difference was not observed among non-RA controls. In contrast, Schönland, *et al*[12] did not observe telomere length differences by *HLA-DRβ1* status among patients with RA. Instead, healthy donors without RA who were carriers of HLA-DRβ1*04 alleles had shorter telomere lengths compared with noncarriers ($p \leq 0.004$). This difference appeared restricted to T cells of individuals in young adulthood because no difference in telomere length by carrier status was observed in T cells of newborns or sperm of healthy men.

Because immune changes can be detected up to 10 years prior to RA onset[39], and studies demonstrate upregulated telomerase in RA synovial tissue and activated T cells (Yamanishi, 1999 #2184; Yudoh, 1999 #2168}, we stratified analyses by length of time between blood collection and RA diagnosis to rule out a transient increase in telomere length in women with preclinical RA at blood collection. RA risk was elevated for the longest versus shortest LTL tertile among women who donated blood samples in all time periods prior to their diagnosis, suggesting our results are not an artifact of reverse causation.

Strengths of our investigation include the largest number of cases examined to date and LTL measured in blood samples collected at least 3 months prior to onset of RA symptoms. We used a modified version of the qPCR-based assay[40], which has been used successfully to identify genetic and disease associations[41,42]. We did not observe significant correlations between age and LTL. The magnitude of the age correlations with LTL in our study was similar to a prior analysis we conducted among 4250 NHS women[26], suggesting lack of power in this limited age range. Factors related to socioeconomic status, smoking, alcohol intake, or BMI were also not correlated with LTL among controls in our study. While LTL has been associated with BMI and/or smoking in some studies[43,44,45], others (including our prior paper) observed nominal or no significant associations[26,46,47,48,49]. We observed heterogeneity between cohorts for the intermediate LTL category and used random-effects metaanalysis to account for this. We recognize the composition of our cohorts (mostly older, white women in health professions) limits generalizability, a tradeoff specifically chosen to maximize the cohorts' internal validity.

Prior studies (Supplemental Table 3, available online at jrheum.org) examined telomere length among T cell subsets and whole blood[8,12,13], but to our knowledge, none have examined B cells or other leukocytes[8,12,13]. We examined telomere length in buffy coat genomic DNA, but could not fractionate blood samples by cell type. Relative changes in telomere length in different white blood cell components in the pre-RA period would be interesting to investigate in future studies. Additionally, studies with more than 1 banked blood sample from the pre-RA period may allow the evaluation of telomere length across the evolution of preclinical to postclinical RA and could provide insight into the involvement of telomeres in RA development.

Our study does not support an involvement for short LTL in RA development. Given the inconsistency with prior literature, our observation of long LTL associated with RA risk may be attributable to chance. Our study highlights the importance of measuring prediagnostic biomarkers to assess whether mechanisms are involved prior to versus during RA pathogenesis. Because our participants are primarily of European ancestry, we were unable to examine LTL associations with RA risk in minority populations. Racial[20,21,22] and sex[50] differences in telomere dynamics prevent the generalizability of our results to non-white or male populations. Therefore, work in diverse populations is warranted to further the understanding of telomere biology in RA pathogenesis.

ACKNOWLEDGMENT

We thank the participants and staff of the Nurses' Health Studies and the Women's Health Study for their valuable contributions.

ONLINE SUPPLEMENT

Supplementary data for this article are available online at jrheum.org.

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