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A Prospective Study Investigating Pre-diagnostic Leukocyte Telomere Length and Risk of Developing Rheumatoid Arthritis in Women

Jennifer Prescott, PhD, Elizabeth W. Karlson, MD, Esther H. Orr, BS, Robert Y.L. Zee, BDS, PhD, Immaculata De Vivo, PhD, MPH, and Karen H. Costenbader, MD, MPH

Channing Division of Network Medicine, and the Division of Rheumatology, Allergy, and Immunology, and Division of Preventive Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, USA; Program in Genetic Epidemiology and Statistical Genetics, Department of Epidemiology, Harvard TH Chan School of Public Health, Boston, Massachusetts, USA; Department of Pediatric Dentistry, Tufts University School of Dental Medicine, Boston, Massachusetts, USA

Abstract

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

Methods—Using a case-control design nested within the prospective Nurses' Health Study (NHS), Nurses' Health Study II (NHSII), and Women's Health Study (WHS), each validated case of RA with a pre-diagnostic blood sample was matched to three 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 odds ratios (OR) and 95% confidence intervals (CI), and random-effects meta-analysis 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. Meta-analysis demonstrated that longer pre-diagnostic LTL was associated with increased RA risk when women in the longest vs. shortest LTL tertile were compared (OR=1.51, 95% CI=1.03–2.23; $P_{\text{heterogeneity}}=0.27$). However, statistically significant between study heterogeneity was observed for the intermediate tertile category ($P_{\text{heterogeneity}}=0.008$). We did not observe heterogeneity by

Address manuscript correspondence to: Jennifer Prescott, PhD, Channing Division of Network Medicine, Brigham and Women's Hospital/Harvard Medical School, 181 Longwood Ave, 3rd floor, Boston, MA 02115 USA, Tel: 617-525-0344, Fax: 617-525-2008, nhjxp@channing.harvard.edu.

Highest academic degrees: J. Prescott, PhD; E.H. Orr, BS; I. De Vivo, PhD, MPH, Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, and Program in Genetic Epidemiology and Statistical Genetics, Department of Epidemiology, Harvard TH Chan School of Public Health; E.W. Karlson, MD; K.H. Costenbader, MD, MPH, Division of Rheumatology, Allergy, and Immunology, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School; R.Y.L. Zee, BDS, PhD, Division of Preventive Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, and Department of Pediatric Dentistry, Tufts University School of Dental Medicine

Address reprint requests to: Karen H. Costenbader, MD, MPH, Division of Rheumatology, Immunology and Allergy, PBB-B3, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115 USA, Tel: 617-732-5158, Fax: 617-713-3030, kcostenbader@partners.org

menopausal status, inflammatory cytokine levels, age at diagnosis, age at blood collection, body mass index, seropositivity, or *HLA-DRB1* shared epitope status.

Conclusion—Our results do not support a role for short LTL preceding RA development.

Key indexing terms

telomere; arthritis; rheumatoid; 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 naïve 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 age⁴. Decline in T- and B-cell function weakens the immune system's ability to recognize "self" vs. "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}.

RA patients exhibit evidence of premature aging. Young RA patients (age <40) were found to have T cell telomere lengths, a marker of proliferative capacity^{6, 7}, similar to participants >65 years of age⁸. Extensive systemic inflammation during active RA, including elevated levels of tumor necrosis factor (TNF)- α , interferon- γ ^{9, 10}, and interleukin (IL)-6¹¹, may secondarily shorten telomeres. However, Weyand and colleagues did not find telomere length differences in patients by disease duration, activity, or severity⁸. Shorter telomeres and proliferation defects were noted in granulocytes¹² and hematopoietic progenitor cells¹³ compared to age-matched controls. Furthermore, healthy individuals positive for major histocompatibility complex, class II, DR beta 1 (*HLA-DRB1*) 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 inflammation¹².

Prospective epidemiologic studies of pre-diagnostic leukocyte telomere length (LTL) as a predictor of incident RA risk have not yet been performed, and could strengthen the argument for a fundamental role of telomere dysfunction in RA development. We used a nested, case-control approach among women from the prospective Nurses' Health Study (NHS), Nurses' Health Study II (NHSII), and Women's Health Study (WHS), to examine whether short pre-diagnostic 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-DRB1* shared epitope carriers).

MATERIALS AND METHODS

Study populations

The NHS is a prospective cohort following 121,700 female U.S. registered nurses aged 30–55 in 1976. NHSII began in 1989 and enrolled 116,608 female U.S. registered nurses aged 25–42. 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 is a completed, randomized, double-blinded, placebo-controlled trial of aspirin and vitamin E in the primary prevention of cancer and cardiovascular disease^{14, 15}. Beginning in September 1992, 39,876 US female health professionals, predominantly white (>94%), aged 45 years and free of cancer or cardiovascular disease enrolled in the study. Participants completed a detailed baseline questionnaire inquiring about their medical history and potential risk factors for cancer and cardiovascular disease. 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 employed a rigorous two-step procedure, described in detail elsewhere^{16, 17}, to validate self-reported physician-diagnosed RA based on the 1987 American College of Rheumatology classification criteria¹⁸. Each cohort utilized a nested case-control design in which validated RA cases were included if they had donated a blood sample, were free of cancer (except non-melanoma 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 three months prior to onset of the first RA symptom documented in the medical record. Presence of rheumatoid factor (RF) and anti-cyclic 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 1990's 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 samples¹⁹.

For each validated case, we randomly selected three women who donated a blood sample, were free of cancer (except non-melanoma 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 year), blood collection characteristics, menopausal status, and recent postmenopausal hormone therapy (HT) use (<3 vs. ≥ 3 months). Premenopausal women also were matched for timing of blood sample within the menstrual cycle. Additionally, WHS participants were matched for length of follow-up since randomization¹⁶. 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 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, MA.

Laboratory assays

LTL in NHS/NHSII genomic DNA samples was assayed as previously described²³. 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 (CVs) for triplicates were <1%. LTL CVs 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 CVs >20% (N=74; 8% of total).

LTL in WHS genomic DNA samples was determined using a previously described quantitative PCR (qPCR)-based protocol²⁴, which differed from the qPCR method used by NHS/NHSII. Telomere and single-copy gene amplifications were done in duplicate on the same 384-well plate. Ct-value assignment was carried out by two independent observers, and if necessary, a complete re-amplification 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 enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN). We measured soluble tumor necrosis factor receptor II (sTNFRII) using a quantitative sandwich ELISA. The range of laboratory batch CVs for each biomarker were 0.07 – 17.7% for IL-6 and 4.0 – 13.6% for sTNFRII. In NHS/NHSII, anti-CCP antibodies were tested via a second generation bead-based microarray using beads coated with peptide antigen (BioRad Laboratories, Hercules, CA). A fluorescence value of >1,500 was considered anti-CCP positive as this cutoff was determined to have similar sensitivity/specificity to plate-based anti-CCP2 ELISAs. Anti-CCP assays were not performed in the WHS due to 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 reported¹⁶.

HLA-DRB1 genotyping of NHS/NHSII samples Genomic DNA was whole-genome amplified with GE Healthcare Genomiphi (GE Healthcare Bio-Sciences Corp., Piscataway, NJ). Low-intermediate resolution *HLA-DRB1* typing was performed on whole-genome amplified DNA using the LABType® SSO (One Lambda, Canoga Park, CA) DNA typing method. Laboratory personnel were blinded to case-control status. Concordance for blinded samples was 100%.

Statistical Analysis

Because 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 procedure²⁵, 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 odds ratios (ORs) and 95% confidence intervals (CIs) 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 NHS/NHSII, we assessed paternal age at birth as a potential confounder as 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 standard deviation LTL variable was modeled as continuous to calculate Wald’s test for trend. Heterogeneity between studies was calculated using the Q statistic, and DerSimonian and Laird meta-analysis was used to combine study results²⁸. We conducted stratified analyses by menopausal status (pre- vs. postmenopausal at blood draw), time between blood draw and diagnosis (<4, 4-<8, 8+ years), IL-6 levels (below vs. above study-specific median), sTNFRII levels (below vs. above study-specific median), median age at diagnosis (<60, 60+ years), age at blood collection (<=50, >50 years), BMI (normal vs. overweight/obese), and *HLA-DRB1* shared epitope status (0 vs. 1 or 2 alleles), and tested interactions via 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 rank sum test compared LTL by seropositivity and *HLA-DRB1* shared epitope status. All p-values are two-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 exposures²⁹. We used SAS Version 9.3 software (SAS Institute, Cary, NC) for all analyses except polytomous logistic regression, which used STATA Version 12.1 software (StataCorp LP, College Station, TX).

RESULTS

We present selected age-adjusted characteristics by study (NHS/NHSII, WHS) and disease status in Table 1. Pre-diagnostic blood samples collected from NHS/NHSII cases occurred at a slightly younger age (52.0 years) than for WHS cases (55.4 years). Due to a longer interval between blood collection and RA diagnosis for NHS/NHSII cases (8.5 years) compared to WHS cases (5.8 years), the mean age at diagnosis was similar between studies (60.5 vs. 61.3 years). 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 sTNFR2, a proxy for the rapidly degraded inflammatory cytokine TNF α , and IL-6 were slightly higher in pre-RA cases compared to 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, sTNFR2, 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 vs. shortest LTL tertile had an OR=1.75 of developing incident RA (95% CI=1.16–2.62; $p_{\text{trend}} = 0.03$; 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 meta-analysis, pre-diagnostic LTL increased RA risk comparing the longest vs. shortest LTL tertile (OR=1.51, 95% CI=1.03–2.23; $p_{\text{heterogeneity}}=0.27$), and the per-standard deviation estimate (OR=1.18, 95% CI=1.03–1.35; $p_{\text{trend}}=0.02$; $p_{\text{heterogeneity}}=0.85$). 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).

As RA risk factors may differ for premenopausal compared to 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 ($p_{\text{heterogeneity}}=0.70$). For those with longest vs. 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; $p_{\text{trend}}=0.05$; $p_{\text{heterogeneity}}$ by time since blood draw=0.85; Table 4). Risk of RA associated with LTL did not differ between women with high vs. low plasma IL-6 ($p_{\text{heterogeneity}}=0.74$) or sTNFR2 levels ($p_{\text{heterogeneity}}=0.94$), by median age at diagnosis ($p_{\text{heterogeneity}}=0.34$), age at blood collection ($p_{\text{heterogeneity}}=0.76$), or BMI ($p_{\text{heterogeneity}}=0.92$) (data not shown).

Prior studies observed significant differences in LTL by RF positivity⁸ or *HLA-DRB1* shared epitope carrier status¹². In our study, LTL did not significantly differ by serostatus among NHS/NHSII ($p=0.76$) or WHS pre-RA cases ($p=0.08$). Meta-analysis of polytomous logistic regression results revealed somewhat stronger risk for seropositive RA (OR=1.74, 95% CI=1.15–2.64; $p_{\text{trend}}=0.01$) than seronegative RA (OR=1.15, 95% CI=0.48–2.76; $p_{\text{trend}}=0.76$) associated with the longest LTL tertile, but the test for heterogeneity was not significant ($p=0.37$; Supplementary Table 1). Risk associated with the longest LTL tertile

did not differ by *HLA-DRB1* shared epitope carrier status ($p=0.45$; Supplementary Table 2). 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 three 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 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 vs. 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. As 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 due to differences in study design and population (Supplementary Table 3). 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 RA patients exhibited shorter telomere lengths across hematopoietic cell lineages compared to controls^{8, 12, 13, 32}. Work conducted by Weyand and colleagues suggested shorter telomeres were restricted to young RA patients (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 group³². 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's lymphoma (NHL) risk by study design. A retrospective study of newly diagnosed aggressive NHL cases observed significantly higher risk associated with short telomeres³³, whereas two independent prospective studies observed significantly increased NHL risk associated with longer pre-diagnostic LTL^{34, 35}.

RA cases from academic medical centers may represent the more severe end of the disease spectrum compared to patients managed in the community³². Our case population, from cohorts of U.S. women, may capture a broader distribution of RA severity compared to prior studies. RA patients with greater disease severity are more likely RF-positive³⁶ and carriers of *HLA-DRB1* 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 shortest, LTL tertile was associated with increased seropositive RA risk. LTL did not differ by seropositivity among these pre-RA cases, consistent with one prior study³², but not another⁸ among established RA patients.

We did not observe significant heterogeneity in the association between LTL and RA risk by *HLA-DRB1* shared epitope carrier status. Elevated risk was associated with the longest vs.

shortest LTL tertile in carriers and non-carriers, and LTL did not differ by carrier status in our study. In Steer et. al.³², RA patients who were carriers had shorter LTL than non-carriers, but this difference was not observed among non-RA controls. In contrast, Schonland et. al.¹² did not observe telomere length differences by *HLA-DRB1* status among RA patients. Instead, healthy donors without RA who were carriers of *HLA-DRB1*04* alleles had shorter telomere lengths compared to non-carriers (p 0.004). This difference appeared restricted to T cells of individuals in young adulthood, as no difference in telomere length by carrier status was observed in T cells of newborns or sperm of healthy men.

As immune changes can be detected up to 10 years prior to RA onset³⁹, 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 pre-clinical RA at blood collection. RA risk was elevated for the longest vs. 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 three months prior to onset of RA symptoms. We used a modified version of the quantitative PCR-based assay⁴⁰, 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 this study was similar to a prior analysis we conducted among 4,250 NHS women²⁶, suggesting lack of power in this limited age range. Factors related to socioeconomic status, smoking, alcohol intake or BMI also were 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 meta-analysis to account for this. We recognize the composition of our cohorts (mostly older, white women in health professions) limits generalizability, a trade-off specifically chosen to maximize the cohorts' internal validity.

Prior studies (Supplemental Table 3) 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 one banked blood sample from the pre-RA period may allow evaluation of telomere length across the evolution of pre-clinical to post-clinical RA and could provide insight into the role of telomeres in RA development.

In conclusion, our study does not support a role for short LTL in RA development. Given the inconsistency with prior literature, our observation of long LTL associated with RA risk may be due to chance. Our study highlights the importance of measuring pre-diagnostic biomarkers to assess whether mechanisms are involved prior to versus during RA pathogenesis. Since 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 sex⁵⁰ 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 understanding of telomere biology in RA pathogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Age-standardized characteristics of participants prior to blood draw by study

Characteristic	Nurses' Health Studies		Women's Health Study	
	Cases N=222	Controls N=584	Cases N=74	Controls N=243
Age at blood draw (years) ^a	52.0 (7.8)	51.6 (8.0)	55.4 (7.7)	55.4 (7.5)
Age at RA diagnosis (years) ^a	60.5 (9.9)	NR	61.3 (7.7)	NR
Time to RA onset (years) ^a	8.5 (4.9)	NR	5.8 (2.5)	NR
Seropositive (RF and/or anti-CCP) %	59	NR	58	NR
Radiographic changes ^b	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 years of college	36	33		
4-year 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

Characteristic	Nurses' Health Studies		Women's Health Study	
	Cases N=222	Controls N=584	Cases N=74	Controls N=243
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 leukocyte telomere length ^c	0.62	0.59	0.24	0.21

Mean (standard deviation) unless otherwise stated

^a Value is not age-adjusted

^b Erosions or periarticular osteopenia consistent with RA

^c Different protocol and calibrator DNA used in Nurses' Health Studies vs. Women's Health Study

Table 2

Association of LTL with incident RA risk by cohort^a

	Nurses' Health Studies			Women's Health Study			Meta-analysis		
	Ca/Co	OR	95% CI	Ca/Co	OR	95% CI	OR	95% CI	p-het
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 standard deviation ^b		1.19	1.01 – 1.40		1.15	0.88 – 1.51	1.18	1.03 – 1.35	0.85
p-trend ^c		0.03			0.30			0.02	

Ca=cases; Co=controls; OR=odds ratio; CI=confidence interval

^aUnconditional logistic regression adjusted for matching factors: age (continuous), menopausal status/HT use at blood draw (premenopausal, menopausal, postmenopausal-no HT, postmenopausal-current HT, unknown), cohort (only for NHS, NHSII),

^bNurses' Health Study LTL standard deviation = 0.26; Women's Health Study LTL standard deviation = 0.71

^cBased on per standard deviation analysis

Association of LTL with incident RA risk by menopausal status in the Nurses' Health Studies and the Women's Health Study combined^a

Table 3

	Premenopause			Postmenopause			p-het
	Ca/Co	OR	95% CI	Ca/Co	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 ^d	0.24 – 4.01	
Longest tertile	35/81	1.73	0.94 – 3.17	64/155	1.45	0.90 – 2.32	
Per standard deviation ^b		1.13	0.89 – 1.43		1.18	0.97 – 1.42	
p-trend ^c		0.31			0.10		0.70

Ca=cases; Co=controls; OR=odds ratio; CI=confidence interval

^a Meta-analysis used to combine estimates from unconditional logistic regression analyses adjusted for the same covariates listed in Table 2

^b Nurses' Health Study LTL standard deviation = 0.26; Women's Health Study LTL standard deviation = 0.71

^c Based on per standard deviation analysis

^d Significant heterogeneity between studies (p=0.01)

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^a

Table 4

	< 4 years			4 - < 8 years			8+ years			p-het
	Ca/Co	OR	95% CI	Ca/Co	OR	95% CI	Ca/Co	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 standard deviation ^b		1.20	0.91 – 1.59		1.14	0.87 – 1.48		1.22	1.00 – 1.49	
P-trend ^c		0.19			0.35			0.05		0.85

Ca=cases; Co=controls; OR=odds ratio; CI=confidence interval

^a Meta-analysis used to combine estimates from unconditional logistic regression adjusted for the same covariates listed in Table 2

^b Nurses' Health Study LTL standard deviation = 0.26; Women's Health Study LTL standard deviation = 0.71

^c Based on per standard deviation analysis