ORIGINAL ARTICLE

Is Chronic Asthma Associated with Shorter Leukocyte Telomere Length at Midlife?

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Abstract

Rationale: Asthma is prospectively associated with age-related chronic diseases and mortality, suggesting the hypothesis that asthma may relate to a general, multisystem phenotype of accelerated aging.

Objectives: To test whether chronic asthma is associated with a proposed biomarker of accelerated aging, leukocyte telomere length.

Methods: Asthma was ascertained prospectively in the Dunedin Multidisciplinary Health and Development Study cohort (n = 1,037) at nine in-person assessments spanning ages 9–38 years. Leukocyte telomere length was measured at ages 26 and 38 years. Asthma was classified as life-course-persistent, childhood-onset not meeting criteria for persistence, and adolescent/adult-onset. We tested associations between asthma and leukocyte telomere length using regression models. We tested for confounding of asthma-leukocyte telomere length associations using covariate adjustment. We tested serum C-reactive protein and white blood cell counts as potential mediators of asthma-leukocyte telomere length associations.

Measurements and Main Results: Study members with life-course-persistent asthma had shorter leukocyte telomere length as compared with sex- and age-matched peers with no reported asthma. In contrast, leukocyte telomere length in study members with childhood-onset and adolescent/adult-onset asthma was not different from leukocyte telomere length in peers with no reported asthma. Adjustment for life histories of obesity and smoking did not change results. Study members with life-course-persistent asthma had elevated blood eosinophil counts. Blood eosinophil count mediated 29% of the life-course-persistent asthma-leukocyte telomere length association.

Conclusions: Life-course-persistent asthma is related to a proposed biomarker of accelerated aging, possibly via systemic eosinophilic inflammation. Life histories of asthma can inform studies of aging.

Keywords: asthma; telomere; aging; longitudinal; developmental phenotype

Asthma is a common, chronic syndrome responsible for substantial health and economic burden in children, adults, and increasingly, older adults (1–3). In adulthood, asthma is characterized by significant comorbidity with other chronic conditions (4); is prospectively associated with risk for developing chronic obstructive pulmonary disease (5–7), cardiovascular

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At a Glance Commentary

Scientific Knowledge on the Subject: Asthma is increasingly recognized as a disease of aging. A possible link between asthma and aging is leukocyte telomere length, a proposed biomarker of cellular aging.

What This Study Adds to the Field: Childhood-onset asthma that persists through midlife is related to shorter leukocyte telomere length, possibly via systemic eosinophilic inflammation. As of midlife, adolescent/adult-onset asthma was not associated with shorter leukocyte telomere length.

disease (8–10), and cancer (11–13); and substantially increases risk for early mortality (14, 15). These observations suggest the hypothesis that asthma may relate to a general, multisystem phenotype of accelerated aging. Here we test the relationship between persistent asthma and one aging indicator, telomere length.

Leading molecular theories of aging identify telomere length as a potential biomarker of cellular aging and as a hypothesized mechanism in the aging process (16, 17). Telomeres are protective caps at the ends of chromosomes that erode with each cell division and thus provide a "biologic clock" tracking cellular aging. In animal studies, early life telomere length is predictive of lifespan (18). *In vitro* studies show a link between telomere shortening and cellular senescence leading to growth arrest (19). In humans, there are reports that shorter leukocyte telomere length is associated with increased morbidity and early mortality (20) and leukocyte telomere length has been proposed as a measure of decline in physiologic integrity across multiple systems (16). Although telomeres remain a controversial biomarker of the aging process (21), leukocyte telomere length provides a useful outcome to test the hypothesis that asthma is associated with accelerated aging for two reasons. First, individual differences in telomere length have been observed early in adult life (22), after individuals have developed asthma but before age-related diseases onset. This allows the isolation of chronic asthma as a correlate of telomere erosion independent of associated comorbidities. Second, chronic asthma is known to affect airway structure and function (23–25). Measurement of telomeres in blood leukocytes allows for a test of asthma's physiologic correlates outside the lung.

Asthma is a developmentally heterogeneous syndrome. Although asthma symptoms often manifest first early in childhood, asthma can commence at any age. The course of asthma is similarly variable, with some cases characterized by full or intermittent remission and others by life-course persistence of symptoms. Sir William Osler is quoted as referring to "asthmatics panting into old age," but asthma may also be associated with reduced life expectancy (14, 15). The extent to which timing of onset and course of asthma are related to aging processes is uncertain. Previous studies of asthma and aging have focused on samples of individuals ascertained in late adulthood. Prospective life-course studies are needed that can distinguish asthma cases based on timing of onset and persistence of disease (26).

In adulthood, asthma may develop secondary to other health problems, including smoking and obesity (27, 28). To disentangle asthma from aging-related features of these other health problems, data are needed that observe the onset and course of asthma from childhood and that can account for potential confounding conditions that confer risk for asthma and accelerated aging.

If asthma is associated with shorter telomere length, this will raise the question of how the relationship comes about. Is it that short telomeres at the beginning of life create vulnerability to asthma? Or does asthma causes damage at the cellular level, resulting in shorter telomeres? In either case, asthma would be involved in aging, although implications for intervention might differ. The key initial step approached by this paper is to test for the asthma-telomere association and to describe the features of the asthma phenotype involved.

We tested associations between asthma and leukocyte telomere length using prospective data from a population-representative birth cohort followed over their first four decades of life, in whom development of asthma has been prospectively ascertained by follow-up at nine assessments at 2- to 6-year intervals from ages 9 to 38 years (29, 30). We measured mean relative leukocyte telomere length in genetic samples obtained at age 26 and again at age 38 years. We tested how the timing of asthma onset and asthma persistence related to telomere length, hypothesizing that the most chronic form of asthma would show the strongest relation to telomere measures. To determine whether associations between asthma and telomere length were attributable to factors that could cause asthma and shorter telomeres in leukocytes, we applied statistical adjustments for histories of obesity and smoking. Finally, we examined how the relationship between asthma and telomere length might be related to inflammation, measured in peripheral blood, the same tissue from which telomeres were assayed.

Methods

Sample

We used data from members of the Dunedin Multidisciplinary Health and Development Study, a longitudinal investigation of health and behavior in a complete (unselected) birth cohort. Study members (1,037; 91% of eligible births; 52% male) were all individuals born between April 1972, and March 1973, in Dunedin, New Zealand, who were eligible for the longitudinal study on the basis of residence in the province at age 3 years and who participated in the first follow-up assessment at age 3 years. The cohort represents the full range of socioeconomic status in the general population of New Zealand's South Island and is mainly white (31). Assessments were done at birth and ages 3, 5, 7, 9, 11, 13, 15, 18, 21, 26, 32, and, most recently, 38 years, when 961 (95%) of the 1,007 surviving study members took part. At each assessment wave, study members are brought to the Dunedin research unit for a full day of interviews and examinations. The Otago Ethics Committee approved each phase of the study and informed consent was obtained.

Measures

Mean relative leukocyte telomere length. Leukocyte DNA was extracted from blood using standard procedures (32, 33). Age-26

and age-38 DNA was stored at −80°C until assayed, to prevent degradation of the samples. All DNA samples were assayed for leukocyte telomere length at the same time, independently of asthma diagnosis. Study members who never developed asthma and study members with different courses of asthma were randomly distributed across different plates. All operations were performed by a laboratory technician masked to asthma status. Leukocyte telomere length was measured using a validated quantitative polymerase chain reaction method (34), as previously described (35), which determines mean telomere length across all chromosomes for all cells sampled. The method involves two quantitative polymerase chain reactions for each subject, one for a single-copy gene (S) and the other in the telomeric repeat region (T). All DNA samples were run in triplicate for telomere and single-copy reactions at ages 26 and 38 (i.e., 12 reactions per study member).

Measurement artifacts (e.g., differences in plate conditions) may lead to spurious results when comparing leukocyte telomere length measured on the same individual at different ages. To eliminate such artifacts, we assayed DNA triplicates from the same individual, from ages 26 and 38, on the same plate. The average coefficient of variation for the triplicate Ct values was 0.81% for the telomere (T) and 0.48% for the single-copy gene (S), indicating high precision. Leukocyte telomere length, as measured by T/S ratio, was normally distributed (Kolomogorov-Smirnov tests of normality), with a skew of 0.90 and kurtosis 1.59 at age 26, and a skew of 0.48 and kurtosis 0.38 at age 38. T/S ratio was transformed to have mean = 0, SD = 1 within age for all analyses (T/S ratio Z-score). Telomere measurements were made in 883 study members of European ancestry who consented to phlebotomy. These individuals formed the analysis sample.

Asthma. We constructed developmental phenotypes of asthma from prospective data collected at nine in-person assessments spanning ages 9–38 years, as previously described (29, 30). Detailed asthma assessments were introduced at age 9 years. At each assessment, study members with a reported diagnosis of asthma and at least one of (*1*) recurrent wheeze, (*2*) asthma attack, or (*3*) asthma medication use in the past year were classified as having current asthma. By age 38 years, 34% of the cohort (n = 352 of 1,037 cohort members; 306 of 883 with telomere data) had been diagnosed with asthma. Asthma persistence was measured as the number of assessments at which study members met criteria for current asthma.

Based on age at onset and persistence, study members with asthma were categorized into three groups. First, we identified cases with onset in childhood and persistence in childhood through midlife. Specifically, this "life-course-persistent" asthma group was defined as having current asthma at two or more assessments up to puberty (age 13 yr) and at three or more assessments thereafter (by age 38 yr, n = 102; 97 with telomere data) (29). Of the life-course-persistent group, half (n = 51) met criteria for current asthma at all their adult assessments. Of the remainder, 23 met criteria for current asthma at five adult assessments, 15 at four assessments, and 13 at three assessments. Study members with asthma who did not meet life-course-persistence criteria were divided into a group with asthma onset in childhood who did not meet criteria for persistence, the childhood-onset group (n = 108; 86 with telomere data), and a group with asthma onset after age 13 years, hereafter the adolescent/adult-onset group (n = 139; 120 with telomere data).

Potential confounders. Review of published literature identified three potential confounders of associations between asthma and leukocyte telomere length: (*1*) socioeconomic disadvantage, (*2*) obesity, and (*3*) cigarette smoking (28, 36–42). We measured cohort members' socioeconomic status as defined from the occupation of their parents when they were children (43). Obesity was measured from anthropometric data at ages 5, 7, 9, 11, 13, 15, 18, 21, 26, 32, and 38 years. Obesity was defined at ages 5–15 as body mass index exceeding the 90th percentile of the sex-specific US Centers for Disease Control and Prevention reference distribution and thereafter as body mass index of 30 or greater (44). At each adult follow-up, we calculated the cumulative number of assessments at which a cohort member had been obese, hereafter "life-course cumulative obesity." Smoking history was assessed during clinical interviews from age 15 onward. These data were used to measure cumulative cigarette consumption in pack-years (a pack-year represents the number of cigarettes consumed during a year spent smoking 20 cigarettes per d) (45).

Inflammation. The Dunedin study took measures of inflammation from peripheral blood at the age-26, -32, and -38 assessments. High-sensitivity assays of C-reactive protein (hsCRP) were conducted at the age-32 and -38 assessments on a Hitachi 917 analyzer (Roche Diagnostics, GmbH, Mannheim, Germany) using a particle-enhanced immunoturbidimetric assay. hsCRP values were log-transformed for analysis. White blood cell (WBC) counts were measured at ages 26, 32, and 38 years (including counts of neutrophils, lymphocytes, monocytes, eosinophils, and basophils) on a fully automated hematology analyzer (Sysmex Corporation, Kobe, Japan). All WBC counts were measured as $\times 10^9$/L and log-transformed for analysis.

Analyses of WBC counts focused on eosinophil and neutrophil counts because these are associated with asthmatic inflammation in lung (46, 47). Peripheral blood eosinophil and neutrophil levels have been questioned as indicators of active airway inflammation, but these cell counts are elevated in patients with asthma (48, 49). Eosinophils are implicated in the pathogenesis of many age-related diseases (50); and eosinophils secrete substances that cause oxidative stress (51) and inhibit telomerase activity (52), processes linked with shorter leukocyte telomere length (53, 54). Peripheral blood neutrophil levels are elevated in chronic obstructive pulmonary disease (55), which is linked with short telomeres (56). Analyses of other WBC counts are presented for purposes of comparison.

Analysis

We analyzed the continuous measure of leukocyte telomere length using regression models. Because telomere length was measured at two adult assessments (when study members were aged 26 and 38 yr), we analyzed data as one longitudinal panel including repeated observations of individuals. These analyses treated each telomere length assessment as an outcome. Generalized estimating equations were used to account for the nonindependence of repeated observations (57). We also conducted a change analysis in which telomere length at age 38 was the

outcome and telomere length at age 26 was included as a covariate. An asthma coefficient from this model indexes the difference in telomere change in asthma cases as compared with cases without asthma.

Asthma phenotypes were defined according to the age at which telomere length was assessed. For example, a cohort member first identified with asthma at age 32 years would be counted as an adolescent/adult-onset asthma case for analysis predicting age-38 telomere length, but doing so was not appropriate for analysis predicting age-26 telomere length. Similarly, asthma persistence was defined as the number of assessments at which the individual met criteria for current asthma up to the particular age of telomere assessment. We included chronologic age and sex as model covariates because asthma prevalence and persistence change over time and vary between men and women (29, 30). We also included as a covariate a product term for the age-sex interaction. We included this covariate first because females more commonly onset with asthma in adulthood as compared with males (who more commonly onset with asthma in childhood) (58) and this is also true in the Dunedin cohort (29); and second because some studies report sex differences in telomere-length change over time (59).

We tested how associations between asthma and telomere length were related to inflammation using generalized estimating equation models and the structural equations described by Baron and Kenny (60) and the methods described by Preacher and colleagues (61, 62).

All biomarker values (leukocyte telomere T/S ratio, hsCRP level, and WBC counts) were standardized for analyses to have mean = 0, SD = 1. Figure 1 and Table 1, which present data showing asthma-telomere length associations, report telomere length in T/S ratio units. All analyses were conducted using Stata 13.0 (StataCorp, College Station, TX) (63).

Results

We first tested whether study members who had developed asthma (of any phenotype) manifested shorter leukocyte telomeres at ages 26 and 38 years as compared with their same-aged peers who had not developed asthma. Study members with ever-diagnosed asthma had shorter telomeres as compared with those in the nonasthma control group, but the result was on the margin of statistical significance (B = −0.12; $P = 0.050$). We next tested the hypothesis that telomere length would be shorter among specifically those cohort members with lifelong chronic asthma (as opposed to all cohort members with asthma). Only cohort members with life-course-persistent asthma had shorter telomere length across age-26 and -38 assessments (B = −0.31; $P < 0.001$). In contrast, there were no differences in telomere length between childhood-onset cases not meeting criteria for persistence and control subjects (B = 0.09; $P = 0.343$) and between adolescent/adult-onset cases and control subjects (B = −0.12; $P = 0.122$). Figure 1 shows average telomere length at ages 26 and 38 years within groups defined by course of asthma.

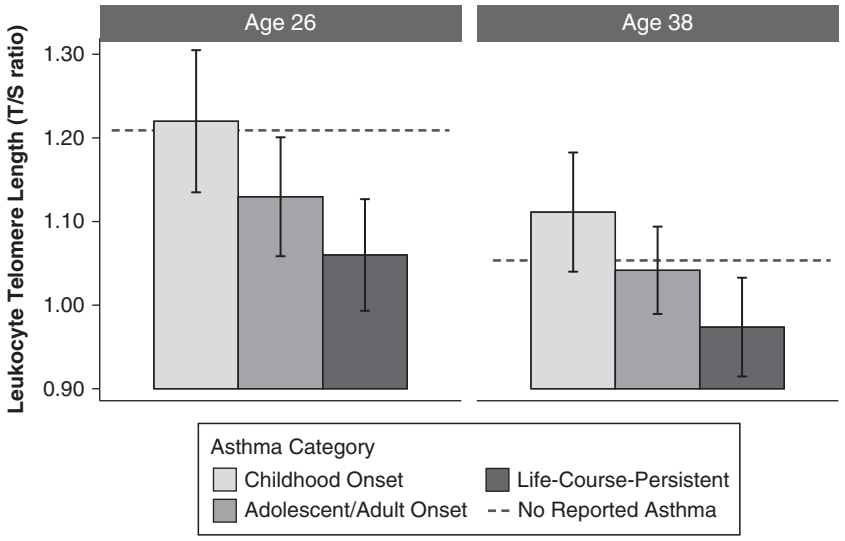

Figure 1. Leukocyte telomere length in cohort members with childhood-onset asthma, adolescent/adult-onset asthma, and life-course-persistent asthma at ages 26 and 38 years. Bar graph average leukocyte telomere length (in T/S ratio units) within groups defined by course of asthma (childhood-onset, n = 86; adolescent/adult-onset, n = 120; and life-course-persistent, n = 97). Error bars show 95% confidence intervals. The *dashed lines* show average leukocyte telomere length in cohort members with no history of asthma. For further information, *see* Table 1.

The developmental phenotypes of asthma that we analyzed describe different patterns of asthma (timing of onset and course of persistence) across the first four decades of life. Because these are descriptive groupings of cases rather than diagnostic categories, we conducted sensitivity analyses. First, we tested whether the persistence of asthma (number of assessments with current asthma) was associated with shorter leukocyte telomere length. Among asthma cases with onset by age 13 years (n = 186), increasing asthma persistence predicted shorter telomere length (B = −0.05; $P = 0.020$), consistent with our analysis of childhood-onset and life-course-persistent asthma groups. Among asthma cases with onset after age 13 years (n = 120), there was no association between asthma persistence and telomere length (B = 0.04; $P = 0.426$). This result suggests that a truly persistent course of asthma across childhood is important to asthma-telomere associations. Second, some of the 97 study members who were classified as life-course-persistent asthma cases did not meet criteria for current asthma at every assessment during adult follow-up (ages 15–38). Restricting the life-course-persistent group to only those cases who always met current asthma criteria did not change results (for the group always meeting current asthma criteria, B = −0.34, $P = 0.001$; for all other life-course-persistent cases, B = −0.30, $P = 0.009$). Hence, childhood-onset asthma cases with a generally persistent course of disease in adulthood but who sometimes presented with no past-year asthma symptoms also manifested shorter telomeres.

Table 1. Leukocyte Telomere Length by Asthma Category

	Age 26 yr				Age 38 yr			
	No Reported Asthma	Childhood-Onset	Adolescent/Adult-Onset	Life-Course-Persistent	No Reported Asthma	Childhood-Onset	Adolescent/Adult-Onset	Life-Course-Persistent
Mean	1.21	1.22	1.13	1.06	1.05	1.11	1.04	0.97
95% Confidence interval	1.17–1.24	1.14–1.30	1.06–1.20	0.99–1.13	1.03–1.08	1.04–1.18	0.99–1.09	0.91–1.03

To test for confounding of the association between life-course-persistent asthma and leukocyte telomere length, we reestimated the association between life-course-persistent asthma and telomere length excluding individuals who grew up in low socioeconomic status households (B = −0.33; $P < 0.001$), who had ever been obese (B = −0.35; $P < 0.001$), and who had ever smoked (B = −0.32; $P = 0.027$). In addition, we repeated regression analyses in the full sample adding statistical adjustment for childhood socioeconomic status, life-course cumulative obesity, and smoking pack-years. Adjustment for these variables did not change the association between life-course-persistent asthma and telomere length (B = −0.31; $P < 0.001$ in adjusted models).

To test whether life-course-persistent asthma cases were experiencing more rapid telomere erosion between ages 26 and 38 years as compared with cohort members without asthma, we fitted a change model: we regressed age-38 telomere length on life-course-persistent asthma status and telomere length at age 26 years. Change in telomere length over this 12-year period was similar in the life-course-persistent asthma cases and in cohort members without asthma (B = 0.05; $P = 0.568$), suggesting that the asthma-telomere association had emerged before age 26, our initial telomere measurement.

Finally, we investigated how the association between life-course-persistent asthma and shorter leukocyte telomere length was related to indicators of inflammation in peripheral blood. Cohort members with life-course-persistent asthma exhibited elevated blood eosinophils as compared with cohort members without asthma (B = 0.96; $P < 0.001$). Blood hsCRP and other WBC levels in cohort members with life-course-persistent asthma were similar to those in cohort members who had not developed asthma. Figure 2 shows differences in peripheral blood levels of hsCRP, and WBC counts in childhood-onset, adolescent/adult-onset, and life-course-persistent asthma cases as compared with individuals who had not developed asthma by the time of assessment. Higher levels of blood eosinophils were associated with shorter telomere length (B = −0.10; $P < 0.001$). After partialing out variance attributable to eosinophils, life-course-persistent asthma remained associated with telomere length, although the effect was attenuated (B = −0.24; $P = 0.005$). The structural model indicated that blood eosinophil count accounted for 29% (95% confidence interval, 15–61%) of the association between life-course-persistent asthma and telomere length. Details for structural models are presented in the online supplement.

Discussion

In this study, we found evidence for association between chronic asthma and shorter leukocyte telomere length in adulthood. Shorter telomeres were found in those with life-course-persistent asthma, but not in childhood-onset or adolescent/adult-onset asthma. Sensitivity analyses confirmed that the association between asthma and shorter telomere length was present only in cases with persistent asthma during childhood and adulthood. This result suggests a mechanism that accumulates throughout development. Shorter telomeres among cohort members with life-course-persistent asthma were not caused by differences in life history of obesity or smoking and were not accounted for by childhood socioeconomic position. Life-course-persistent asthma did not predict a more rapid rate of telomere change between ages 26 and 38 years. One interpretation of this result is that that whatever process links chronic asthma and telomere length has already occurred by young adulthood. Alternatively, we may not have detected change in telomere length within the life-course-persistent asthma group because of right-hand censoring (our follow-up ends at age 38 yr). Finally, our data are agnostic as to the causal direction of the asthma-telomere association. However, whatever the causal direction of the association, systemic eosinophilic inflammation seems to be involved. Specifically, increased levels of circulating eosinophils accounted for just under one-third of the association between chronic asthma and telomere length.

The pathogenesis of many age-related diseases involves eosinophils (50), which secrete substances that cause oxidative stress (51) and inhibit telomerase activity (52) (processes linked with shorter leukocyte telomere length [53, 54]). If eosinophilic inflammation causes short telomere length during early stages of innate immune development, short telomeres should be characteristic of eosinophilic disorders of childhood. If the process requires chronic exposure, short telomere length may not be observed until later in life.

We acknowledge limitations. First, left censoring of telomere measurements means our study cannot establish the causal ordering of chronic asthma and shorter leukocyte telomeres. Future studies with measurements of telomeres beginning early in childhood can help to clarify whether short telomeres precede asthma onset or if the onset and persistence of asthma shortens telomeres. Second, right censoring of all measurements leaves open the possibility that cases of chronic asthma will come to have telomeres of similar length to asthma-free individuals, or that other groups (e.g., adult-onset asthma cases) will experience more rapid telomere erosion and come to resemble the life-course-persistent cases. Continued follow-up

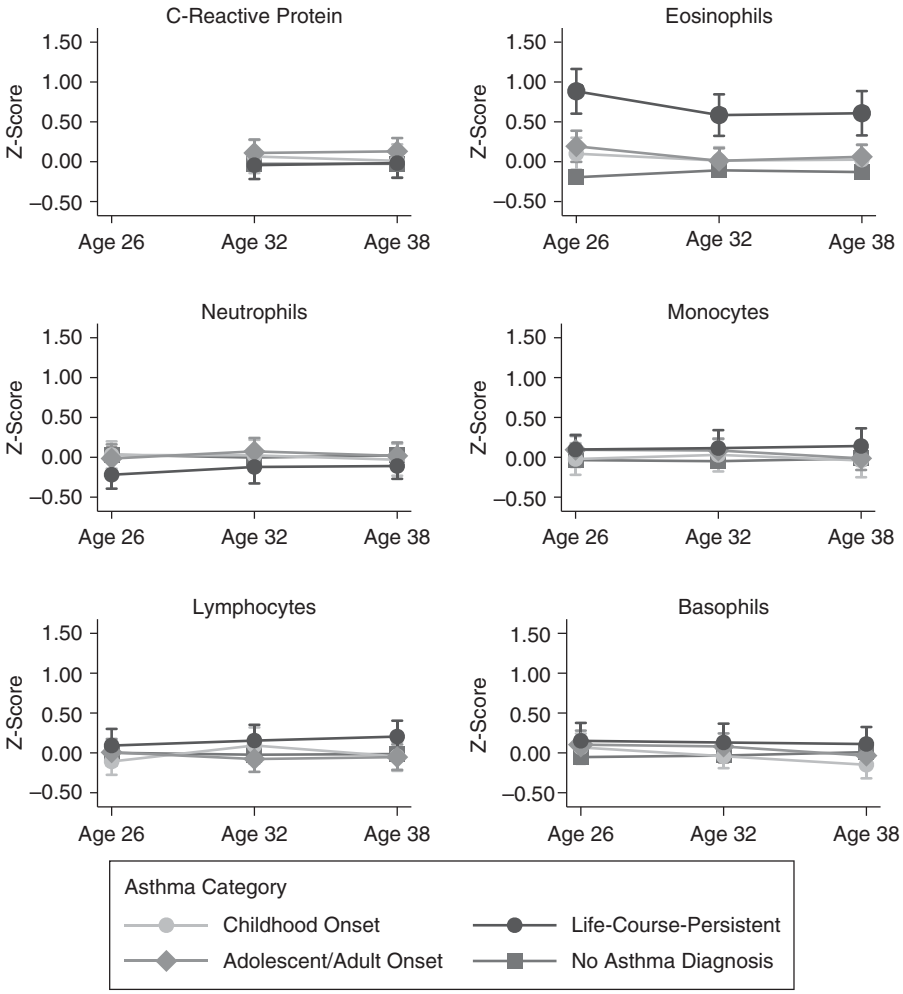

Figure 2. Serum levels of C-reactive protein and counts of eosinophils, neutrophils, monocytes, lymphocytes, and basophils at ages 26, 32, and 38 years among cohort members with childhood-onset asthma, adolescent/adult-onset asthma, and life-course-persistent asthma. Biomarker levels are graphed in terms of standard deviations from cohort means (*z* scores). High-sensitivity assays of C-reactive protein were conducted at the age 32 and 38 assessments only. Only eosinophils differed in the life-course-persistent asthma group as compared with individuals with no reported asthma (B = 0.96; $P < 0.001$). This difference was statistically significant after correcting for multiple testing (Bonferroni corrected, $P < 0.001$). A box plot illustrating eosinophil data in more detail is included in the online supplement.

of this cohort and further research in other cohorts that track the natural history of adult asthma are needed. Studies including follow-up into the second half of the life course can examine how comorbid health conditions and medications affect asthma-telomere associations and the role of asthma and short telomere length in age-related decline in lung function. From our analysis, asthma seems to relate to shorter telomere length only in cases characterized by onset in childhood and a persistent course, because shorter telomeres were not observed in childhood-onset cases without persistence and telomere length was not related to the persistence of asthma among those with onset in adolescence or adulthood.

Third, our cohort was from a single country and was primarily of European descent. Replication in other populations and in other countries is needed. Finally, although our analyses implicate systemic eosinophilic inflammation in the association between asthma and telomere length, we lack cell-type–specific measures of telomere length. If short telomere length confers refractory inflammation, it is important to know whether this is a cell-autonomous phenotype. Determining whether short telomeres are characteristic of all component cell types within leukocytes could inform understanding of mechanism. We also lack measures of inflammation from sputum or airway biopsies. Lower levels of human telomerase reverse transcriptase expression in submucosa of bronchial biopsies of patients with asthma have been reported (64). Research is needed to characterize mechanisms linking asthma and telomere length.

Our study constitutes an incremental advance in research on asthma and aging. To our knowledge, only two previous studies have tested associations between asthma and leukocyte telomere length (64, 65). As with previous studies, we find an association between asthma and shorter leukocyte telomere length. Our findings from a large, population-based birth cohort followed over four decades indicate that the link between asthma and telomere length is most pronounced in individuals with a childhood-onset, persistent course of asthma. Furthermore, the link between this phenotype of life-course-persistent asthma and telomere length is related to elevated systemic eosinophilic inflammation.

An implication of these findings is that life histories of asthma can inform studies of aging. First, studies of asthma and telomere length in particular, and of asthma and aging more generally, should seek to distinguish asthma cases on the basis of course of disease (early onset and subsequent persistence). Second, because asthma often begins early in life and persistent asthma is associated with poor health outcomes in aging, future studies investigating telomere-length correlations with specific age-related disease (e.g., chronic obstructive pulmonary disease [56]) should consider participants' life histories of asthma. Finally, although asthma has traditionally been studied as a disease of childhood, studies of adult asthma and studies linking asthma with multimorbidity in later life have highlighted asthma as a disease of aging. Future studies of the aging process may benefit from information about participants' histories of asthma. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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Chronic Asthma and Leukocyte Telomere Length

Is Chronic Asthma Associated with Shorter Leukocyte Telomere Length at Midlife?

Supplemental Material

Mediation Analysis

We tested mediation using a system of 3 equations:

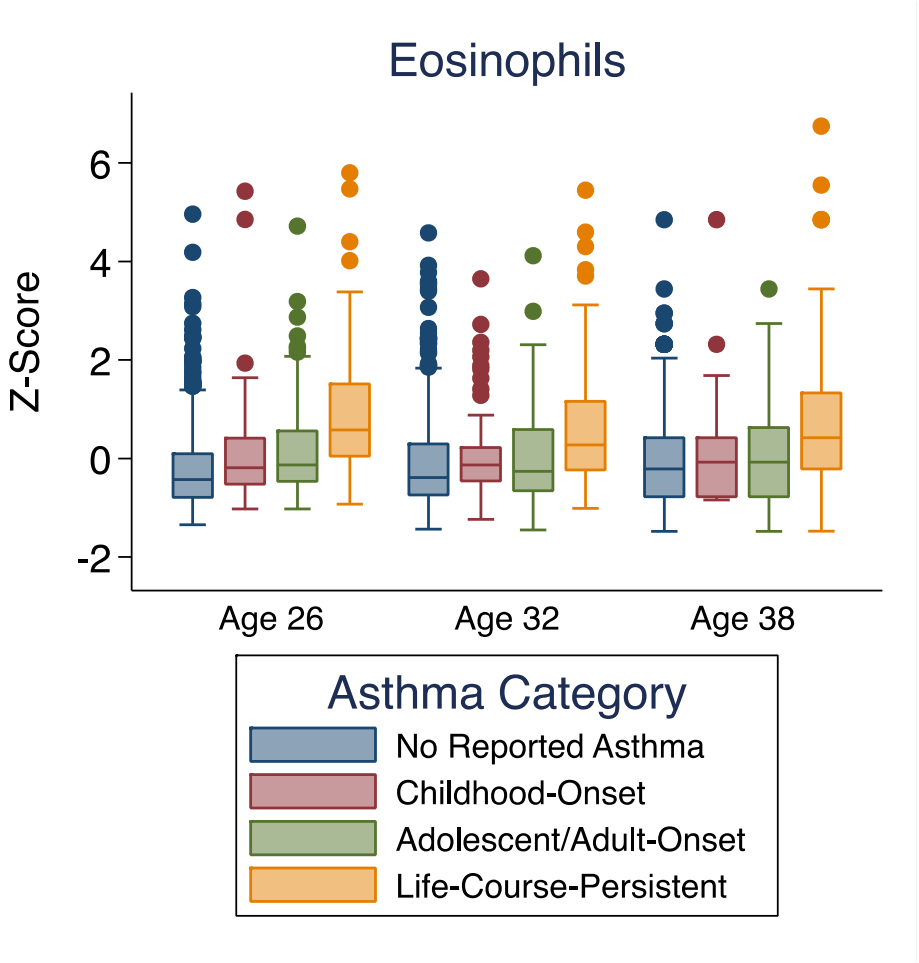
1. *Telomere Length* $= \Upsilon_1 + \tau Asthma + \eta X + \varepsilon_1$
2. *Mediator* $= \Upsilon_2 + \alpha Asthma + \eta X + \varepsilon_2$
3. *Telomere Length* $= \Upsilon_3 + \tau' Asthma + \beta Mediator + \eta X + \varepsilon_3$

The total effect of asthma on telomere length was estimated as τ. The indirect effect of asthma mediated through eosinophil count was estimated as the product of coefficients α and β.[1] Percentile-based confidence intervals for estimates were calculated using the bootstrap method.[2] Estimates of the total, indirect, and direct effects are reported in **Supplemental Table 1**.

Supplemental Table 1. Total, indirect, and direct effect estimates from models testing mediation of associations between life-course-persistent asthma and leukocyte telomere length at ages 26 and 38 years by blood eosinophil count. Total effect estimates reflect the association between life-course-persistent asthma and telomere length. Indirect effect estimates reflect the portion of this total effect overlapping the association of blood eosinophil count with telomere length. Direct effects reflect the residual association between life-course-persistent asthma and telomere length that was independent of blood eosinophil count. Percentile-based 95% Confidence Intervals (CIs) were estimated from 1,000 bootstrap repetitions.

Exposure: Life-Course-Persistent Asthma Outcome: Leukocyte Telomere Length Third Variable: Blood Eosinophil Count		
	Estimate	**Percentile-Based 95% CI**
Total Effect	-0.32	-0.49 , -0.15
Indirect Effect	-0.09	-0.15 , -0.05
Direct Effect	-0.24	-0.41 , -0.07
% of Association Accounted for by Eosinophil Count	29%	0.15 , 0.61

Supplemental Figure 1. Box Plot of Eosinophil Count Z-Score by Age and Asthma Category.



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the parenchymal and airway disease in COPD with much more clarity. Advancements in phenotyping in conjunction with genetics have provided better understanding about the genetic susceptibility in COPD. The fact that several of the GWAS results for lung function and COPD susceptibility are also associated with distinct emphysema patterns is encouraging, whereas the question of whether these loci are associated with COPD or emphysema remains unanswered. Methods for quantification of local emphysema and airway disease patterns are also evolving. This will provide more opportunities to integrate these phenotypes with genetics and genomics for systems biology analyses and determination of molecular phenotypes in COPD. Localization of emphysema on treatment outcomes is also emerging. A large multicenter study comparing lung volume reduction surgery with medical treatment has shown that patients with upper lobe emphysema and low exercise capacity who received the surgery had a greater survival rate than similar patients who received medical therapy (15). In a recent randomized control trial evaluating the efficacy of a γ selective retinoid agonist in the treatment of emphysema, placebo patients with lower lung emphysema deteriorated faster than those with predominantly upper lobe disease. In addition, patients with lower lung emphysema appeared to respond better to the treatment (16). Adding more granularity using LHE and other regional emphysema measurements will definitely help advance this field. This makes us wonder, is COPD like the GOLDen rule of real estate . . . location, location, location? ■

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New Asthma Biomarkers: Shorter Telomeres, Longer Disease?

Asthma is a disease characterized by large variability in its natural history and clinical course. Patients can experience clinical manifestations that go from mild, sporadic wheezing episodes to life-threatening attacks, and anything in between. Once the disease has occurred, the clinical course can follow any combination of persistence, remission, and relapse, with a substantial and hardly predictable interpatient variability. This difficulty in predicting the natural history of asthma and, for that matter, the individual

response to treatment and tertiary prevention is partly related to our limited understanding of the molecular mechanisms that underlie the disease and its sequelae.

In this framework, biomarker research holds the promise—or at least the potential—to provide, on one side, new insights into the molecules and pathways that drive the disease processes and, on the other, to improve our ability to predict individual outcomes, persistence of disease, and, in turn, to "personalize" intervention strategies.

In this issue of the *Journal*, Belsky and colleagues (pp. 384–391) provide an additional contribution to the field by using longitudinal data from the Dunedin birth cohort to investigate the potential role of a newly proposed biomarker of persistent asthma: leukocyte telomere length (1). Telomeres are repetitive DNA sequences located at chromosomal ends that are critical for the maintenance of genomic integrity. Their progressive shortening with cell divisions leads to cellular senescence and apoptotic death and, as such, reduced telomere length has been proposed as a general marker of aging and linked to morbidity and mortality in several degenerative and age-related diseases (2, 3).

Two recent cross-sectional studies (4, 5) have first reported that leukocyte telomere length may also be shorter in subjects with asthma as compared with healthy control subjects and correlate inversely with disease severity. These previous findings are now confirmed and expanded by the study by Belsky and colleagues (1) in at least three ways. First—and most importantly—in this study the association between short telomere length and asthma is investigated within a longitudinal study design. Participants were followed from 9 to 38 years of age, and leukocyte telomere length at ages 26 and 38 years was found to be shorter in the group of subjects who had persistent asthma from childhood into adult age but not among subjects who had childhood asthma that remitted in adulthood or among those who only had adult-onset asthma. These findings suggest one of two possible scenarios: either an accelerated "molecular clock"—which may be influenced by genetic factors and/or early developmental processes—predisposes to an early-onset, chronic form of the disease; or the persistence of active symptoms from childhood into adult life and their related inflammatory processes lead to significant telomere shortening. However, telomere shortening between ages 26 and 38 years was not accelerated in any of the asthma groups as compared with subjects with no asthma. Therefore, the telomere length deficits associated with childhood asthma that persists into adulthood are likely to be established by early adult life, if not in childhood already. No telomere length assessments were available from earlier ages in the Dunedin study, and the conundrum of whether short telomere length precedes or is rather a consequence of persistent asthma will need to be addressed in future studies. By assessing telomere length and asthma phenotypes from the early stages of life and, in turn, linking them to disease outcomes in adulthood, these studies will also contribute to establishing whether leukocyte telomere length can provide any useful information to identify, ahead of time, children with asthma who will go on to have persistent disease as adults.

A second important strength of the study by Belsky and colleagues (1) is the use of a population-based birth cohort with a remarkably low attrition rate. This study design allowed the authors to compare leukocyte telomere length between disease groups within the same age intervals (i.e., at 26 and 38 yr) and, therefore, to minimize the risk of potential confounding by age differences across asthma phenotypes. This issue had not been systematically addressed by previous research in the field and is particularly relevant in light of the established strong relation of aging to telomere length (6, 7). However, the price to be paid for the methodological strengths of this type of cohort study is that molecular investigations usually need to rely on biospecimens that are easy to collect and the least burdensome for participants (i.e., almost invariably blood samples). This was also the case for the Dunedin study. Thus, whether the association between short telomere length of leukocytes and chronic asthma that was found in this study also applies to (or may even be stronger for) other cell types remains to be determined. Previous studies support a direct correlation between telomere length measured in leukocytes and in samples from the lungs, skeletal muscle, skin, subcutaneous fat, and saliva (7–9). However, the strength of this correlation and its relevance in asthma for cells that may be directly involved with disease processes in the airways are unknown. Of note, in patients with chronic obstructive pulmonary disease (COPD), telomere length has been shown to be reduced both in leukocytes and other cells from lung tissue, including alveolar type II and endothelial cells (10). Answering this question in asthma will contribute to elucidating whether the role of telomere shortening in this disease is mediated by mechanisms that are shared across different tissues and whether this biomarker may have any value in molecular phenotyping.

Last but not least, it is worth noting that in the study by Belsky and colleagues (1) both persistent asthma and shorter telomere length were found to be associated with elevated blood eosinophils, suggesting that blood eosinophilia may be involved in the link between the two. This finding holds particular interest because eosinophilia has been shown to characterize the subgroup of subjects with asthma who are at increased risk of developing persistent airflow limitation (11), the hallmark of COPD. Indeed, severity and persistence of asthma—two disease characteristics associated with shorter telomere length (1, 4)—have been consistently linked to worse disease outcomes in terms of lung function deficits. For example, in this same cohort, individuals who had persistent wheezing symptoms between age 9 and 26 years also had the lowest levels of the ratio between FEV_1 and FVC throughout that age range (12). It is therefore tempting to speculate that accelerated aging processes that are reflected by telomere shortening may increase the risk of patients with persistent asthma to develop COPD and, in turn, an overlap syndrome that carries an elevated morbidity and mortality burden (13). Although this scenario is in line with the previously established relation of short telomere length to lung function deficits in asthma (5) and to risk, morbidity, and mortality in COPD (3, 14, 15), at the present time it remains an untested hypothesis.

Indeed, as evidence for the relation of leukocyte telomere length to asthma has begun to build up, many of the above questions will need to be tested before the robustness and possible clinical implications of this association can be established and before some, undoubtedly needed, light can be shed on its nature and implicated mechanisms. ■

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The Child Is Father of the Man?

Establishing the origins of diseases such as asthma is one of the most important goals of research today.

Asthma affects hundreds of millions of people worldwide. It is the most frequent disease in childhood for which parents visit their doctors, yet the origins of asthma are still mainly unclear. In past decades, epidemiologic studies have provided better insights into the etiology of asthma and provided several risk factors that can contribute to this airway disease. Both developmental risk factors in utero and in early childhood, such as environmental tobacco smoke exposure, and genetic factors contribute to disease development, and these risk factors may interact (1).

Early childhood risk factors in the first years of life are especially important during the time of rapid lung development and growth. During that period, all children are exposed to viruses that are inhaled in the respiratory tract and that can affect epithelial cells, underlying tissues, and the immune system. As a consequence, many respiratory wheezing episodes occur in that time of life after an early-life lower respiratory illness (LRI). It has been shown that these LRIs, especially when induced by respiratory syncytial virus (RSV), can be followed by asthma-like symptoms (2), and later on, by a physician diagnosis of asthma with additional lung function measurements in childhood (3), a risk that tends to diminish toward adolescence (4, 5). This risk is especially increased in children with severe RSV-LRI who needed hospitalization in early life (6).

Gern and Busse distinguished two nonexclusive relationships between RSV-LRI and wheezing (7). They postulated that RSV bronchiolitis, as can occur after RSV infection, may interfere with normal lung development or immune maturation. This then leads to recurrent episodes of wheezing. Alternatively, RSV infection might constitute the first stimulus for wheezing in children who are predisposed to wheeze by genetic susceptibility or preexisting abnormal lung function at birth (7). However, observational studies cannot determine whether RSV infection is the cause of recurrent wheeze or the first indication of preexistent pulmonary vulnerability in preterm infants. Therefore, a prospective study was designed by Blanken and colleagues (8). A double-blind study with palivizumab, an RSV immunoprophylactic agent, during the RSV season showed that active treatment resulted in a significant reduction in wheezing days during the first year of life in preterm children, a finding that remained present even after the end of treatment. These findings implicate RSV infection as an important causal mechanism of recurrent wheeze during the first year of life in such infants. It remains to be determined whether these protective effects on wheeze are also present in term infants at risk for the development of asthma; a study to investigate this was recently recommended (9).

Of interest, wheezing episodes after an RSV-LRI have been shown to reduce by adolescence, suggesting this is a childhood risk only (3–6). This also would suggest that RSV-LRI is not an asthma risk but, instead, a wheezing risk in the first decade of life. In this issue of the *Journal*, Voraphani and colleagues (pp. 392–398) showed that this is indeed the case; that is, objectified RSV-LRI in children of the Tucson birth cohort followed up to 29 years of age did not relate to an increased risk for asthma at that age when RSV-LRI had taken place in the first years of life (10). However, the authors