AJRCCM Articles in Press. Published on 23-June-2014 as 10.1164/rccm.201402-0370OC

Chronic Asthma and Leukocyte Telomere Length

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

Daniel W. Belsky, PhD.[1,2]

Idan Shalev, Ph.D.[3]
Malcolm R. Sears, M.B.[4]
Robert J. Hancox, M.D.[5]
HonaLee Harrington, B.Sc.[6]
Renate Houts, Ph.D.[6]
Terrie E. Moffitt, Ph.D.[6-9]
Karen Sugden, Ph.D.[8]
Benjamin Williams, B.Sc.[8]
Richie Poulton, Ph.D.[5]
Avshalom Caspi, Ph.D.[6-9]

1. Center for The Study of Aging and Human Development, Duke University Medical Center, Durham, NC, USA
2. Social Science Research Institute, Duke University, Durham, NC, USA
3. Department of Biobehavioral Health, Pennsylvania State University, State College, PA, USA
4. Division of Respirology, Department of Medicine, DeGroote School of Medicine, McMaster University, Hamilton, ON, Canada
5. Department of Preventive and Social Medicine, School of Medicine, University of Otago, New Zealand
6. Department of Psychology & Neuroscience, Duke University, Durham, NC, USA
7. Department of Psychiatry and Behavioral Sciences, School of Medicine, Duke University, Durham, NC, USA
8. Institute for Genome Sciences and Policy, Duke University, Durham, NC, USA
9. Social, Genetic, and Developmental Psychiatry Centre, Institute of Psychiatry, Kings College London, London, UK

Address Correspondence to
Daniel W. Belsky
2020 W. Main St. Suite 201, Durham NC 27708
Tel. 919-613-4534
Fax 919-684-6679
Email dbelsky@duke.edu

Author Contributions: DWB and IS conceived the study; MRS, RJH, TEM, RP, and AC collected the data; DWB, IS, AC, RH, HLH, BW, and KS analyzed the data; DWB, IS, AC, RJH, MRS, and TEM wrote the manuscript; all authors provided critical review of the manuscript and approved its submission.

Acknowledgment. We thank the Dunedin Study members, their families, unit research staff, and the Dunedin Study founder. The Dunedin Multidisciplinary Health and Development Research Unit is supported by the New Zealand Health Research Council. Collection of respiratory data was supported in part by the Otago Medical Research Foundation and the New Zealand Asthma Foundation. This research received support from US National Institute on Aging (NIA) grant AG032282 and UK Medical Research Council grant MR/K00381X. Additional support was provided by the Jacobs Foundation. DWB received support from the NIA through a postdoctoral fellowship T32 AG000029 and P30 AG028716-08.

Running Title: Chronic Asthma and Leukocyte Telomere Length

Descriptor Number: 1.14

At a Glance Commentary: In this longitudinal study of a population-based birth cohort followed over 4 decades, asthma persisting from childhood through midlife was associated with shorter leukocyte telomere length at midlife.

This article has an online data supplement, which is accessible from this issue's table of content online at www.atsjournals.org

Word Count: 3,463
Abstract Word Count: 242

Copyright © 2014 by the American Thoracic Society

Chronic Asthma and Leukocyte Telomere Length

ABSTRACT

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

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

Method. Asthma was ascertained prospectively in the Dunedin Multidisciplinary Health and Development Study cohort (N=1,037) at 9 in-person assessments spanning ages 9 to 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.

Results. Study members with life-course-persistent asthma had shorter leukocyte telomere length as compared to 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.

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 23-June-2014 as 10.1164/rccm.201402-0370OC

INTRODUCTION

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 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, multi-system phenotype of accelerated aging. Here we test the relations 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 an 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 “biological 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 physiological 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

Copyright © 2014 by the American Thoracic Society

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 physiological correlates outside the lung.

Asthma is a developmentally heterogeneous syndrome. While 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 both 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-

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 23-June-2014 as 10.1164/rccm.201402-0370OC

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 9 assessments at 2-6 year intervals from ages 9-38 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 both 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 (1037; 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

Copyright © 2014 by the American Thoracic Society

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 1007 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 carried out by a laboratory technician blinded to asthma status. Leukocyte telomere length was measured using a validated quantitative PCR method,[34] as previously described,[35] which determines mean telomere length across all chromosomes for all cells sampled. The method involves two quantitative PCR 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 both 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 both ages 26 and 38, on the same plate. The average coefficient of variation

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 23-June-2014 as 10.1164/rccm.201402-0370OC

(CV) 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 9 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 (a) recurrent wheeze, (b) asthma attack, or (c) 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 years) and at three or more assessments thereafter (by age 38 years, 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

Copyright © 2014 by the American Thoracic Society

remainder, 23 met criteria for current asthma at 5 adult assessments, 15 at 4 assessments, and 13 at 3 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: socioeconomic disadvantage, obesity, and 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 onwards. 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 day).[45]

Inflammation. The Dunedin study took measures of inflammation from peripheral blood at the

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 23-June-2014 as 10.1164/rccm.201402-0370OC

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, D-68298, 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 haematology analyser (Sysmex Corporation, Japan). All WBC counts were measured as $x10^9$/L and log-transformed for analysis.

Analyses of WBC counts focused on eosinophil and neutrophil counts as 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 asthma patients.[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 years), we analyzed data as one longitudinal panel including repeated observations of individuals. These analyses treated each telomere length assessment as an

Copyright © 2014 by the American Thoracic Society

outcome. Generalized estimating equations were used to account for the non-independence 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 to 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 chronological 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 firstly because females more commonly onset with asthma in adulthood as compared to males (who more commonly onset with asthma in childhood)[58] and this is also true in the Dunedin cohort;[29] and secondly 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 et al.[61,62]

All biomarker values (leukocyte telomere T/S ratio, hsCRP level, and white blood cell counts) were standardized for analyses to have mean=0 SD=1. The figure depicting asthma-

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 23-June-2014 as 10.1164/rccm.201402-0370OC

telomere length associations reports telomere length in T/S ratio units. All analyses were conducted using Stata 13.0.[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 to their same-aged peers who had not developed asthma. Study members with ever-diagnosed asthma had shorter telomeres as compared to those in the non-asthma 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 controls (B=0.09, p=0.343) and between adolescent/adult-onset cases and controls (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.

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

Copyright © 2014 by the American Thoracic Society

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.

To test for confounding of the association between life-course-persistent asthma and leukocyte telomere length, we re-estimated 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

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 23-June-2014 as 10.1164/rccm.201402-0370OC

telomere erosion between ages 26 and 38 years as compared to 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 to 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 white blood cell counts in childhood-onset, adolescent/adult-onset, and life-course-persistent asthma cases as compared to 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% CI 15%-61%] of the association between life-course-persistent asthma and telomere length. Details for structural models are presented in the **Supplemental Materials**.

Copyright © 2014 by the American Thoracic Society

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 due to right hand censoring (our follow-up ends at age 38 years). Finally, our data are agnostic as to the causal direction of the asthma-telomere association. But, whatever the causal direction of the association, systemic eosinophilic inflammation appears 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

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 23-June-2014 as 10.1164/rccm.201402-0370OC

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 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 appears to relate to shorter telomere length only in cases characterized by onset in childhood and a persistent course, as 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

Copyright © 2014 by the American Thoracic Society

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 hTERT expression in submucosa of bronchial biopsies of asthma patients 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 4 decades indicate that the link between asthma and telomere length is most pronounced in individuals with a childhood-onset, persistent course of asthma. Further, 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

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 23-June-2014 as 10.1164/rccm.201402-0370OC

asthma with multi-morbidity 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.

Copyright © 2014 by the American Thoracic Society

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. Bars 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.

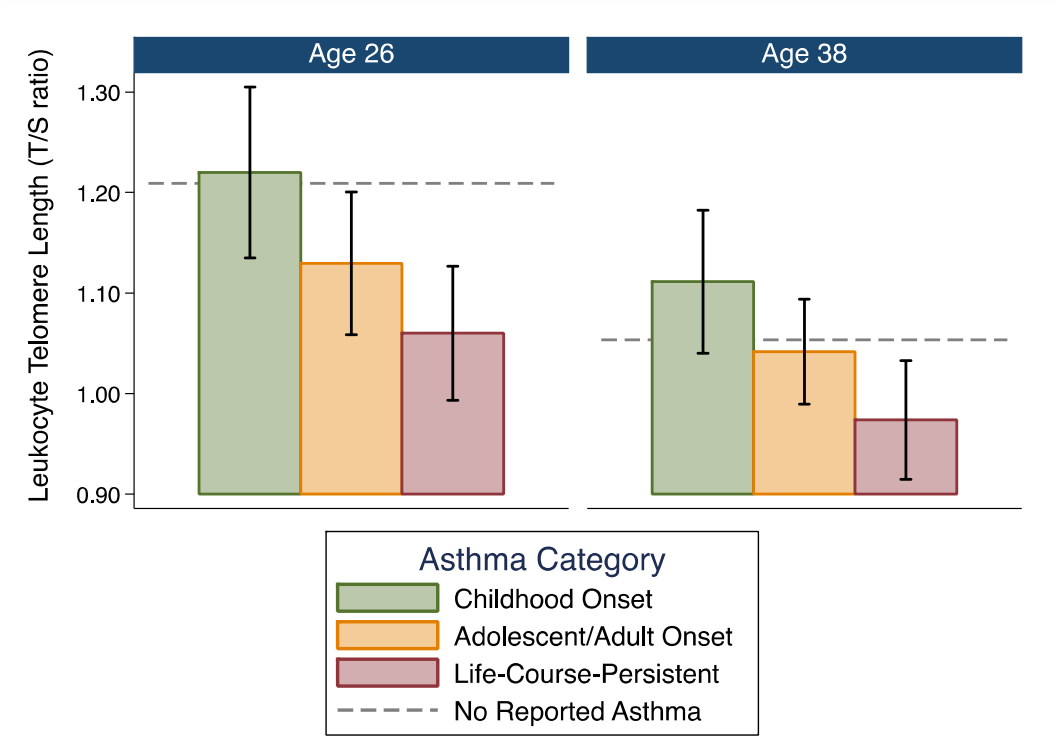
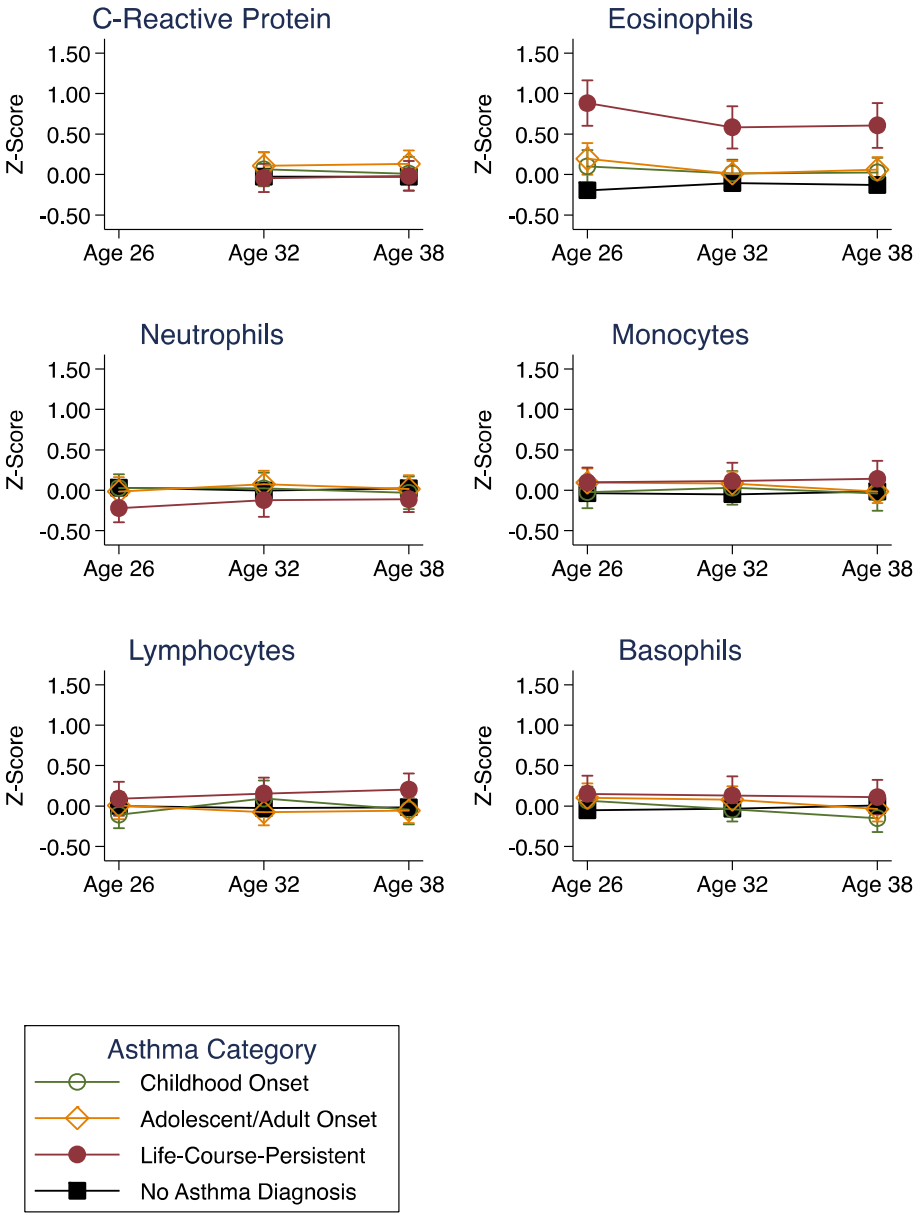


Figure Data: Leukocyte Telomere Length by Asthma Category									
	Age 26					Age 38			
	No Reported Asthma	Childhood-Onset	Adolescent/Adult-Onset	Life-Course-Persistent		No Reported Asthma	Childhood-Onset	Adolescent/Adult-Onset	Life-Course-Persistent
Mean	1.21	1.22	1.13	1.06		1.05	1.11	1.04	0.97
95% CI	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

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 23-June-2014 as 10.1164/rccm.201402-0370OC

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 to individuals with no reported asthma (B=0.96, p<0.001). This difference was statistically significant after correcting for multiple testing (Bonferronni corrected p<0.001). A box plot illustrating eosinophil data in more detail is included in the supplement.



Copyright © 2014 by the American Thoracic Society

REFERENCES

1. Gibson PG, McDonald VM, Marks GB. Asthma in older adults. Lancet 2010;376:803–13.

2. Sullivan PW, Ghushchyan VH, Slejko JF, Belozeroff V, Globe DR, Lin S-L. The burden of adult asthma in the united states: evidence from the medical expenditure panel survey. J Allergy Clin Immunol 2011;127:363–369.e3.

3. Akinbami LJ, Moorman JE, Garbe PL, Sondik EJ. Status of childhood asthma in the united states, 1980-2007. Pediatrics 2009;123:S131–S145.

4. Tsai C-L, Delclos GL, Huang JS, Hanania NA, Camargo CA. Age-related differences in asthma outcomes in the united states, 1988-2006. Ann Allergy Asthma Immunol 2013;110:240–6, 246.e1.

5. Lange P, Parner J, Vestbo J, Schnohr P, Jensen G. A 15-year follow-up study of ventilatory function in adults with asthma. N Engl J Med 1998;339:1194–200.

6. Silva GE, Sherrill DL, Guerra S, Barbee RA. Asthma as a risk factor for copd in a longitudinal study. Chest 2004;126:59–65.

7. Mannino DM, Buist AS. Global burden of copd: risk factors, prevalence, and future trends. Lancet 2007;370:765–73.

8. Hospers JJ, Rijcken B, Schouten JP, Postma DS, Weiss ST. Eosinophilia and positive skin tests predict cardiovascular mortality in a general population sample followed for 30 years. Am J Epidemiol 1999;150:482–91.

9. Knoflach M, Kiechl S, Mayr A, Willeit J, Poewe W, Wick G. Allergic rhinitis, asthma, and atherosclerosis in the bruneck and army studies. Arch Intern Med 2005;165:2521–6.

10. Iribarren C, Tolstykh IV, Miller MK, Sobel E, Eisner MD. Adult asthma and risk of coronary heart disease, cerebrovascular disease, and heart failure: a prospective study of 2 matched cohorts. Am J Epidemiol 2012;176:1014–24.

11. Mayne ST, Buenconsejo J, Janerich DT. Previous lung disease and risk of lung cancer among men and women nonsmokers. Am J Epidemiol 1999;149:13–20.

12. Santillan AA, Camargo CA, Colditz GA. A meta-analysis of asthma and risk of lung cancer (united states). Cancer Causes Control 2003;14:327–34.

13. Rosenberger A, Bickeböller H, McCormack V, Brenner DR, Duell EJ, Tjø nneland A, et al. Asthma and lung cancer risk: a systematic investigation by the international lung cancer consortium. Carcinogenesis 2012;33:587–97.

14. Bellia V, Pedone C, Catalano F, Zito A, Davì E, Palange S, et al. Asthma in the elderly:

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 23-June-2014 as 10.1164/rccm.201402-0370OC

mortality rate and associated risk factors for mortality. Chest 2007;132:1175–82.

15. Ali Z, Dirks CG, Ulrik CS. Long-term mortality among adults with asthma: a 25-year follow-up of 1,075 outpatients with asthma. Chest 2013;143:1649–55.

16. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. Cell 2013;153:1194–217.

17. Newgard CB, Sharpless NE. Coming of age: molecular drivers of aging and therapeutic opportunities. J Clin Invest 2013;123:946–50.

18. Heidinger BJ, Blount JD, Boner W, Griffiths K, Metcalfe NB, Monaghan P. Telomere length in early life predicts lifespan. Proc Natl Acad Sci 2012;109:1743–8.

19. Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. Nature 1990;345:458–60.

20. Armanios M, Blackburn EH. The telomere syndromes. Nat Rev Genet 2012;13:693–704.

21. Mather KA, Jorm AF, Parslow RA, Christensen H. Is telomere length a biomarker of aging? a review. J Gerontol A Biol Sci Med Sci 2011;66A:202–13.

22. Shalev I. Early life stress and telomere length: investigating the connection and possible mechanisms: a critical survey of the evidence base, research methodology and basic biology. BioEssays News Rev Mol Cell Dev Biol 2012;34:943–52.

23. Elias JA, Zhu Z, Chupp G, Homer RJ. Airway remodeling in asthma. J Clin Invest 1999;104:1001–6.

24. Rasmussen F, Taylor DR, Flannery EM, Cowan JO, Greene JM, Herbison GP, et al. Risk factors for airway remodeling in asthma manifested by a low postbronchodilator fev1/vital capacity ratio: a longitudinal population study from childhood to adulthood. Am J Respir Crit Care Med 2002;165:1480–8.

25. Lambrecht BN, Hammad H. The airway epithelium in asthma. Nat Med 2012;18:684–92.

26. Hanania NA, King MJ, Braman SS, Saltoun C, Wise RA, Enright P, et al. Asthma in the elderly: current understanding and future research needs—a report of a national institute on aging (nia) workshop. J Allergy Clin Immunol 2011;128:S4–S24.

27. Appleton SL, Adams RJ, Wilson DH, Taylor AW, Ruffin RE. Central obesity is associated with nonatopic but not atopic asthma in a representative population sample. J Allergy Clin Immunol 2006;118:1284–91.

28. Beuther DA, Sutherland ER. Overweight, obesity, and incident asthma: a meta-analysis of prospective epidemiologic studies. Am J Respir Crit Care Med 2007;175:661–6.

29. Belsky DW, Sears MR, Hancox RJ, Harrington H, Houts R, Moffitt TE, et al. Polygenic risk

Copyright © 2014 by the American Thoracic Society

and the development and course of asthma: an analysis of data from a four-decade longitudinal study. Lancet Respir Med 2013;1:453–361.

30. Sears MR, Greene JM, Willan AR, Wiecek EM, Taylor DR, Flannery EM, et al. A longitudinal, population-based, cohort study of childhood asthma followed to adulthood. N Engl J Med 2003;349:1414–22.

31. Moffitt TE, Caspi A, Rutter M, Silva PA. Sex differences in antisocial behavior: conduct disorder, delinquency, and violence in the dunedin longitudinal study. Cambridge: Cambridge University Press; 2001.

32. Bowtell DDL. Rapid isolation of eukaryotic dna. Anal Biochem 1987;162:463–5.

33. Jeanpierre M. A rapid method for the purification of dna from blood. Nucleic Acids Res 1987;15:9611–9611.

34. Cawthon RM. Telomere measurement by quantitative pcr. Nucleic Acids Res 2002;30:e47–e47.

35. Shalev I, Moffitt TE, Sugden K, Williams B, Houts RM, Danese A, et al. Exposure to violence during childhood is associated with telomere erosion from 5 to 10 years of age: a longitudinal study. Mol Psychiatry 2013;18:576–81.

36. Caudri D, Wijga A, A. Schipper CM, Hoekstra M, Postma DS, Koppelman GH, et al. Predicting the long-term prognosis of children with symptoms suggestive of asthma at preschool age. J Allergy Clin Immunol 2009;124:903–910.e7.

37. Hafkamp-de Groen E, Lingsma HF, Caudri D, Levie D, Wijga A, Koppelman GH, et al. Predicting asthma in preschool children with asthma-like symptoms: validating and updating the piama risk score. J Allergy Clin Immunol 2013;epub ahead of print.

38. Accordini S, Janson C, Svanes C, Jarvis D. The role of smoking in allergy and asthma: lessons from the ecrhs. Curr Allergy Asthma Rep 2012;12:185–91.

39. Valdes a M, Andrew T, Gardner JP, Kimura M, Oelsner E, Cherkas LF, et al. Obesity, cigarette smoking, and telomere length in women. Lancet 2005;366:662–4.

40. Epel ES. Telomeres in a life-span perspective a new “psychobiomarker”? Curr Dir Psychol Sci 2009;18:6–10.

41. Geronimus AT, Hicken MT, Pearson JA, Seashols SJ, Brown KL, Cruz TD. Do us black women experience stress-related accelerated biological aging? Hum Nat 2010;21:19–38.

42. Robertson T, Batty GD, Der G, Fenton C, Shiels PG, Benzeval M. Is socioeconomic status associated with biological aging as measured by telomere length? Epidemiol Rev 2012;:98–111.

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 23-June-2014 as 10.1164/rccm.201402-0370OC

43. Poulton R, Caspi A, Milne BJ, Thomson WM, Taylor A, Sears MR, et al. Association between children's experience of socioeconomic disadvantage and adult health: a life-course study. Lancet 2002;360:1640–5.

44. Belsky DW, Moffitt TE, Houts R, Bennett GG, Biddle AK, Blumenthal JA, et al. Polygenic risk, rapid childhood growth, and the development of obesity: evidence from a 4-decade longitudinal study. Arch Pediatr Adolesc Med 2012;166:515–21.

45. Belsky DW, Moffitt TE, Baker TB, Biddle AK, Evans JP, Harrington H, et al. Polygenic risk and the developmental progression to heavy, persistent smoking and nicotine dependence: evidence from a 4-decade longitudinal study. JAMA Psychiatry 2013;70:534–42.

46. Anderson GP. Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. The Lancet 20;372:1107–19.

47. Wenzel SE. Asthma: defining of the persistent adult phenotypes. The Lancet 368:804–13.

48. Szefler SJ, Wenzel S, Brown R, Erzurum SC, Fahy JV, Hamilton RG, et al. Asthma outcomes: biomarkers. J Allergy Clin Immunol 2012;129:S9–S23.

49. Hastie AT, Moore WC, Li H, Rector BM, Ortega VE, Pascual RM, et al. Biomarker surrogates do not accurately predict sputum eosinophil and neutrophil percentages in asthmatic subjects. J Allergy Clin Immunol 2013;132:72–80.e12.

50. Simon D, Simon H-U. Eosinophilic disorders. J Allergy Clin Immunol 2007;119:1291–300.

51. Babior BM. Phagocytes and oxidative stress. Am J Med 2000;109:33–44.

52. Norrback K-F, Enblad G, Erlanson M, Sundström C, Roos G. Telomerase activity in hodgkin's disease. Blood 1998;92:567–73.

53. Blackburn EH. Switching and signaling at the telomere. Cell 2001;106:661–73.

54. Von Zglinicki T. Oxidative stress shortens telomeres. Trends Biochem Sci 2002;27:339–44.

55. Stockley RA. Neutrophils and the pathogenesis of copd. Chest 2002;121:151S–155S.

56. Rode L, Bojesen SE, Weischer M, Vestbo J rgen, Nordestgaard B rge G. Short telomere length, lung function and chronic obstructive pulmonary disease in 46,396 individuals. Thorax 2013;68:429–35.

57. Zeger SL, Liang KY, Albert PS. Models for longitudinal data: a generalized estimating equation approach. Biometrics 1988;44:1049–60.

Copyright © 2014 by the American Thoracic Society

58. Leynaert B, Sunyer J, Garcia-Esteban R, Svanes C, Jarvis D, Cerveri I, et al. Gender differences in prevalence, diagnosis and incidence of allergic and non-allergic asthma: a population-based cohort. Thorax 2012;67:625–31.

59. Barrett ELB, Richardson DS. Sex differences in telomeres and lifespan. Aging Cell 2011;10:913–21.

60. Baron RM, Kenny DA. The moderator mediator variable distinction in social psychological-research - conceptual, strategic, and statistical considerations. J Pers Soc Psychol 1986;51:1173–82.

61. Preacher KJ, Hayes AF. Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. Behav Res Methods 2008;40:879–91.

62. Preacher KJ, Kelley K. Effect size measures for mediation models: quantitative strategies for communicating indirect effects. Psychol Methods 2011;16:93–115.

63. StataCorp. Stata statistical software: release 13. StataCorp LP; 2013.

64. Kyoh S, Venkatesan N, Poon AH, Nishioka M, Lin T-Y, Baglole CJ, et al. Are leukocytes in asthmatic patients aging faster? a study of telomere length and disease severity. J Allergy Clin Immunol 2013;132:480–482.e2.

65. Albrecht E, Sillanpää E, Karrasch S, Alves AC, Codd V, Hovatta I, et al. Telomere length in circulating leukocytes is associated with lung function and disease. Eur Respir J 2014;43:983–92.

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 23-June-2014 as 10.1164/rccm.201402-0370OC

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**.

Copyright © 2014 by the American Thoracic Society

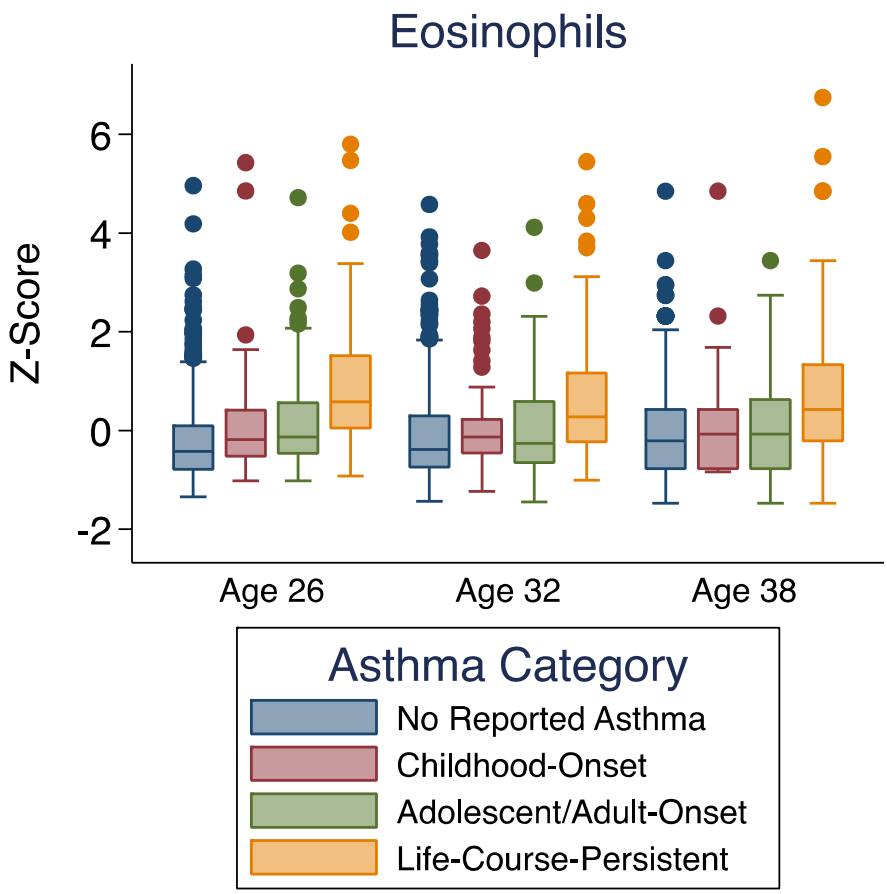
Supplemental Table 1. Total, indirect, and direct effect estimates from models testing mediation of associations between life-course-persistent asthma and leukocyte telomere length at ages 26 and 38 years by blood eosinophil count. Total effect estimates reflect the association between life-course-persistent 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

Copyright © 2014 by the American Thoracic Society

AJRCCM Articles in Press. Published on 23-June-2014 as 10.1164/rccm.201402-0370OC

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



Copyright © 2014 by the American Thoracic Society

REFERENCES

1. Baron RM, Kenny DA. The moderator mediator variable distinction in social psychological-research - conceptual, strategic, and statistical considerations. J Pers Soc Psychol 1986;51:1173–82.

2. Preacher KJ, Hayes AF. Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. Behav Res Methods 2008;40:879–91.

Copyright © 2014 by the American Thoracic Society