Correlation between Telomere Length and COPD-related Phenotypes: results from the chronic obstructive pulmonary disease in dusty areas (CODA) cohort

Da Hye Moon[1], Jeeyoung Kim[2], Myoung-nam Lim[3] , So Hyen Bak[4], Woo Jin Kim[1,2]

[1]Department of Internal Medicine, Kangwon National University Hospital, Chuncheon, Republic of Korea

[2]Department of Internal Medicine and Environmental Health Center, Kangwon National University School of Medicine, Chuncheon, Republic of Korea

[3]Biomedical Research Institute, Kangwon National University, Chuncheon, Republic of Korea

[4]Department of Radiology, Kangwon National University Hospital, Kangwon National University of School of Medicine, Chuncheon, Republic of Korea

Address for correspondence: Woo Jin Kim, M.D., Ph.D.

Department of Internal Medicine, Kangwon National University School of Medicine, 1 Gangwondaehak-gil, Chuncheon 24341, Korea
Phone:: 82-33-258-9364, Fax; 82-33-255-6567

E-mail: pulmo2@kangwon.ac.kr

Abstract

Background: COPD is a common chronic respiratory disease with increased prevalence as older. Telomeres are repetitive DNA sequences found at the end of the chromosome, which progressively shorten as cells divide. Telomere length is known to be a molecular marker of aging. This study aimed to assess the relationship between telomere length and the risk of COPD, lung function, respiratory symptoms, and emphysema index in chronic obstructive pulmonary disease in dusty areas (CODA) cohort.

Methods: We extracted DNA from the peripheral blood samples of 446 participants, including 285 COPD patients and 161 control participants. We measured absolute telomere length using quantitative real-time polymerase chain reaction. All participants underwent spirometry and quantitative computed tomography scan. Questionnaires assessing respiratory symptoms and the COPD Assessment Test (CAT)) was conducted for all participants.

Results: The mean age of participants at the baseline visit was 72.5±7.1 years. Males accounted for 72%(321 participants) of the all participants. The mean telomere length was shorter in the COPD group compared to the non COPD group (COPD; 16.81±13.90, Non-COPD; 21.97±14.43 kb). In COPD patients, 112(75.7%) were distributed as tertile1(shortest), 91 (61.1%) as tertile2 and 82 (55%) as tertile3(longest). We did not find significant associations between telomere length and lung function, exacerbation, airway wall thickness and emphysema index after adjusting for sex, age, and smoking status.

Conclusion: In this study, the relationship between various COPD phenotypes and telomere length was analyzed, but no significant statistical associations were shown.

Keywords : Telomere length, Chronic obstructive pulmonary disease, phenotype

Introduction

Chronic obstructive pulmonary disease(COPD) is a major and increasing global health problem with large healthcare costs[1]. COPD is a heterogeneous disease. It comprises emphysema in the lung parenchyma, large central airway inflammation, mucociliary dysfunction, bronchiolitis, and small airway structural changes[2]. Airflow limitation, measured by reduced forced expiratory volume in 1s (FEV_1), progresses very slowly over several decades. Therefore most patients with symptomatic COPD are in the late middle ages or are elderly. An accelerated rate of lung function decline with age is one of the central pathophysiological characteristics of COPD. Therefore COPD is a prevalent chronic respiratory disease, and its prevalence increases with age [1].

Telomeres are repetitive DNA sequences, with high G-C content, at the end of chromosome protection. They prevent chromosomal ends from being recognized as double-strand breaks and protect them from end-to-end fusion and degradation[3]. DNA polymerases cannot fully replicate chromosome, since one RNA primer remains on each daughter DNA strand. This loss of base pairs is a partial consequence of the so-called end-replication problem[4]. Therefore, DNA cannot be duplicated at the end of the chromosome. Each duplication results in a gradual shortening of telomeres, until a critical length is reached at which point cells undergo apoptosis[5]. Furthermore, exposure to oxidative stress and inflammation aggravates this shortening[6]. Therefore, some studies have shown that telomere length is a biomarker of cellular aging [7,8].

Several studies have shown a significant relationship between reduced telomere length in peripheral blood leukocytes and increased risk of malignancy, cardiovascular disease, and diabetes mellitus[9-11]. In these studies, leukocyte telomere length was used as a biomarker of aging based on the assumption that reflects the physiological age of individuals. Since COPD

is an age-dependent process, assessment of telomere length may be useful in better understanding the pathogenesis of the disease.

Since COPD is a heterogeneous disease with variable phenotypes [12], we assumed that the shorter the telomere length, the more depressed the lung function, and the higher the exacerbation rate and degree of emphysema. Accordingly, this study aimed to assess the relationship between telomere length and lung function, exacerbation, and visual assessments of emphysema as well as smoking exposure in a Korean COPD cohort residing near cement plants.

Materials and Methods

1. Study design and population

Participants in the Chronic Obstructive Pulmonary Disease in Dusty Areas (CODA) cohort were analyzed[13]. The CODA study enrolled participants living near six cement plants in the Kangwon and Chungbuk provinces of South Korea. Overall, 504 participants (362 men and 142 women) were enrolled for baseline examinations between 2012 and 2017(Figure 1). Participants were excluded that 25 with pneumoconiosis, bronchiectasis or destroyed lung on chest CT scans , 4 without a collected questionnaire, 5 without telomere length results and 23 with extreme telomere length results. Finally, 446 participants were eligible.

At baseline examinations, a medical interview and survey questionnaire were administered, and spirometry, physical examination, blood/urine sampling, and computed tomography(CT) were performed for all participants. The questionnaire evaluated demographic factors, lifestyle factors, medical history, exacerbation history, and respiratory symptoms during the past year. We defined moderate exacerbation as a history of antibiotics or steroid use more

than twice, and severe exacerbation as more than one hospitalization due to respiratory symptom within a year.

Written informed consent was given by each participant. This study received ethical approval from the Kangwon National University Hospital IRB (KNUH 2020-06-007).

2. Measurement

Dyspnea was evaluated using the modified Medical Research Council(mMRC) scoring system[14]. Quality of life was assessed using a patients-reported COPD assessment test (CAT).

Spirometry was measured yearly using the Easy One Kit (NDD Medizintechnik AG, Zurich, Switzerland), before and after inhalation of 400-μg salbutamol. All pulmonary function tests were performed according to the guidelines of the American Thoracic Society/European Respiratory Society[15].

3. CT image analysis

All participants underwent volumetric, thin-section, chest CT at full inspiration and expiration in the supine position. CT images were acquired using a first-generation, dual-source scanner (Somatom Definition; Siemens Healthcare, Forchheim, Germany) in the caudocranial direction using the following parameters: 140 kVp, 100mA, 0.9–1 beam pitch and slice thickness of 0.6mm and 3mm. The CT data were reconstructed using a soft convolution kernel (B30f)[16]. Emphysema was evaluated by automatically extracting all lung images from the chest wall, mediastinum and large airways. The attenuation coefficients of pixels in these images were then measured. The emphysema index was defined as the volume fraction (%) of the lung below −950 HU in full inspiration[17]. Mean wall area percentage

(MWA%) was calculated as a percentage of the mean values measured in two segmental bronchi (wall area/[wall area + lumen area] x100)[18]. The visually defined subtype of COPD were evaluated by 2 radiologists (with 3 and 11 years of experience) based on the Fleischner Society classification system[19]. Different interpretations were resolved by consensus. The subtype is classified into following 7 types: (1) normal, (2) paraseptal emphysema (subjects with substantial paraseptal emphysema), (3) bronchial airway disease, (4) trace centrilobular emphysema, (5) mild centrilobular emphysema, (6) moderate centrilobular emphysema, and (7) confluent and advanced destructive emphysema[20].

4. Telomere length measurement

Venous blood samples were obtained at baseline and DNA was extracted from the buffy coat. Telomere lengths was measured in DNA isolated from leukocytes. We modified the Cawthon method for relative measurement of telomere length by introducing an oligomer standard to measure absolute telomere length (aTL). In this approach, aTL was calculated by quantitative polymerase chain reaction (qPCR) according to by O′Callaghan and Frenech method[21], where a standard curve was generated from the fluorescent signals obtained from a series of known concentrations of telomere oligomer DNA(TTAGG X 14). The concentration of each test sample was predicted by plotting the fluorescence signal of the sample onto a standard curve. To serve as a reference gene, the concentration of a single copy gene′s (36B4) DNA in each sample was measured using the same method. Telomere length was calculated as the ratio of telomere DNA length from the standard curve to the 36B4 DNA length.

The telomere lengths of each sample were normalized to a reference cell line control sample, which was evaluated on each plate. Telomere length measurements (kb/genome) were normalized by natural log-transformation. For analysis, we divided the participants into

three tertiles according to their telomere length.

5. Statistical analysis

446 participants divided to COPD and non COPD groups. COPD was defined according to GOLD with post-bronchodilator FEV1/FVC <0.70. For the analysis, the telomere length for each group (all participants, COPD group and non COPD group) was divided into tertile group. Comparison of baseline characteristics between COPD group and non COPD group was performed using a Student′s t-test and Chi-square test. Categorical variable are described as number (%). Continuous variables are reported as the mean±standard deviation. The lung function, mMRC, and CAT score were evaluated using a general linear model (GLM) adjusting for age, sex, smoking status, height. To compare the trend of lung function decline according to the tertile groups of telomere length of telomere length used a mixed model adjusting for age, sex, smoking status. Logistic regression was used to find out the association between telomere length and COPD exacerbation. Odds ratios (ORs) and 95% confidence intervals (CI) were calculated using logistic regression model after adjusting for sex, age, smoking status. For trend tests, individuals were categorized according to telomere length tertile (coded 1-3) with the first tertile consisting of individuals with the shortest telomere lengths. *P*-values less than 0.05 were, considered statistically significant. All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA)

Results

1. Clinical characteristics, respiratory symptoms and lung function of the all

participants and COPD patients

The clinical characteristics, respiratory symptoms and lung function of 446 participants at baseline are summarized in Table1. There were 285 participants (63.9%) in the COPD group and 161 (36.1%) in the non-COPD group. The average telomere length was 18.68±14.29kb. The mean telomere length was shorter in the COPD group compared to the non-COPD group (COPD; 16.81±13.90 kb, Non-COPD; 21.97±14.43 kb). The mean age of participants at the baseline was 72.5±7.1 years and 72% (321 participants) of the cohort were men (Table 1).

We divided the patients into three groups according to telomere length in all participant (tertile 1: < 8.54, tertile 2: 8.54- 23.54, tertile 3: > 23.54) . A total of 285 participants (63.9%) had a FEV1/FVC < 0.70. In COPD patients, 112(75.7%) were distributed as tertile1, 91 (61.1%) as tertile2 and 82 (55%) as tertile3 (Table2). There were no statistically significant associations between lung function and telomere length after adjusting for sex, age, and smoking status. However, shorter telomere length showed the tendencies that decreased FVC at pre-bronchodilator and FVC, FVC % predicted at post-bronchodilator in COPD patients (*P* trend: 0.217, 0.157, and 0.319, respectively) (Table 3). Non-COPD group was showed that shorter telomere length in tertile showed the tendencies that decreased FEV1/FVC at pre-bronchodilator and post-bronchodilator (*P* trend: 0.03 and 0.005, respectively) (Supplement Table 1).

2. The relationship between telomere length and lung function decline

In all participants and COPD patient, we showed the tendencies that shorter telomere length in tertile was associated with decreased FVC L decline at pre-bronchodilator. (p trend: <0.0001, < 0.0001, respectively) (Table 4, Table 5). In non COPD group, longer telomere

length in tertile showed the tendencies that increased FVC L at post-bronchodilator (P trend: < 0.0001) (Supplement Table 2).

3. The relationship between telomere length and visual and quantitative CT imaging features

Of the 446 participants, 25 were excluded from the visual assessment of the CT scans due to severe lung distortion and other lung morbidity. Finally, we analyzed 421 participants. Among the 285 patients with COPD, 13 were excluded from the visual assessment of CT scans. Finally, we analyzed 272 patients. There were no statistically significant association between telomere length and emphysema index, mean wall area and CT subtypes in all participants and COPD patients (Table 6, Supplement Table 3). In non COPD participants, we showed the trend that decreasing telomere length was associated with increased mean wall area (*P* trend: 0.415) (Supplement Table 4).

4. The relationship between telomere length and acute exacerbation

Additionally, we did not find a significant association between telomere length and COPD exacerbation in COPD patients. In multivariable analyses, OR for the shortest versus the longest telomere tertile was 0.707 (95% CI 0.153-3.274) for moderate exacerbation and 0.788 (0.203 to 3.057) for severe exacerbation(Table 7).

Discussion

We investigated the relationship between telomere length and the lung function, respiratory symptoms, emphysema extent and airway wall thickness in a Korean COPD cohort living

near cement plants. Disappointingly, we did not observe significant associations between telomere length and lung function, exacerbation, airway wall thickness and emphysema index after adjusting for sex, age, and smoking status. However, statistical significances have not been shown, some tendencies were shown along the telomere length tertile. There were shown that FEV1/FVC (pre-, post-bronchodilator) decreased with shorter telomere length in all participants and non COPD group. The shorter telomere length was showed that decreased FVC (pre-, post-bronchodilator) in COPD group. In the all participants and COPD patients, the shorter the telomere length, the lower the FVC (pre-bronchodilator) decline in the lung function change. On the other hand, in the non COPD group, the shorter the telomere length , the lower the FVC (post- bronchodilator) increase in the lung function change. In addition, the shorter the telomere length, the wider the mean wall area in non COPD group.

COPD is an age-related disease. An accelerated rate of lung function decline with age is one of the central pathophysiological characteristics of COPD. Previous studies have shown that leukocyte telomeres can be as biomarkers of cellular aging[7,22,23]. Some studies have shown that decreased leukocyte telomere length is associated with a decline in lung function[24,25]. In our study, some tendencies between short telomere length and decreased lung function were shown, but statistical significant association was not shown.

Rode *et al*. reported that FEV_1, FVC and FEV_1/FVC decreased with decreasing telomere length quartile (p trend: $5x\ 10^{-51}$, $5x10^{-35}$ and $6x10^{-137}$, respectively) but the associations attenuated after age and multivariable adjustment. In addition, participants with shorter telomeres were 2.06-fold more likely to develop COPD. The results showed how short telomere length is associated with reduced lung function and an increased prevalence of COPD[6]. Furthermore, data from seven studies demonstrated highly significant positive associations between telomere length and spirometric measurements including FEV_1 (β=0.0455, $p=1.07x10^{-7}$ with fixed and random effects;I^2=0%), FVC (β=0.0401, $p=2.07x10^{-7}$

with fixed and random effects;I^2=0%), and FEV_1/FVC (β=0.0238, p=5.27x10^{-7} with fixed and random effects;I^2=0%)[25].

One study reported that shorter leukocyte telomere length may be a biomarker for associated with a poor clinical prognosis in COPD, where short telomere length was associated with reduced quality of life and increased risk of exacerbation and mortality in patients with moderate-to-severe COPD[26]. However, our study did not show the relationship between acute COPD exacerbation and telomere length.

And no significant associations were found between the emphysema index, mean wall area and telomere length in the all participants and COPD patients (*P* trend: 0.486, 0.669, 0.553, 0.328, respectively). In the sub-analysis, the correlation of the average telomere length and emphysema CT subtype did not show a statistically significant trend in all partcipants and COPD group(*P* trend: 0.179, 0.390, respectively). In addition, we did not observe any association between telomere length and airway wall thickness, emphysema index and visual CT image features in non COPD group. However, we showed the trend that the shorter the telomere length, the wider the mean wall area in non COPD group.

The MESA(Multi-Ethnic Study of Atherosclerosis) study found that the presence of centrilobular and panlobular emphysema corelated with increased dyspnea and reduced exercise capacity[27]. Airway wall thickness correlated with reduced lung function and increased symptoms in smoker in a cross-sectional study[28]. Image biomarkers have been useful predictors of disease progression. As shown earlier, previous studies showed that telomeres were shorter in peripheral leukocytes of COPD patients and have been related with lung function. However, we did not find other studies on the relationship between CT-based visual assessments and telomere length.

Smoking is a well-known environmental factor that promotes aging and cellular

senescence[29]. A large population study showed that a smoking history and the cumulative pack-years were associated with telomere length in 46,396 adults[30]. Another study found that smoking promotes shortening of telomere length. Within the same age range, telomere length decreased with age in smokers, irrespective of the presence or absence of COPD (p=0.05, r= -0.27). The influence of cumulative smoking exposure on telomere length is further supported by the significant dose-effect relationship demonstrated between pack-years and telomere length (p<0.001, r=-0.45)[31]. In this study, when analyzing the relationship between telomere length and pack-year (*P* trend: 0.029, 0.020, respectively), it observed statistical significances, but did not show a certain trend in all participants and COPD patients.

There were some strengths to this study. It is meaningful that this is the first study to examine the relationship between telomere length and the phenotypes of COPD in Koreans. Our study investigated the relationship between telomere length and the extent of emphysema, airway wall thickness and visual assessment on CT scans. Some studies have demonstrated that emphysema as assessed by CT imaging, is a good predictor of mortality in COPD patients at various stages of the disease. Although it is still unclear whether emphysema predisposes COPD patients to such systemic manifestations and whether these systemic manifestations contribute to the development of emphysema, it is apparent that recognizing the extent of emphysema is important when evaluating COPD. Taken together, the evaluation of emphysema seems to be beneficial for the management of COPD[32].

Nevertheless, this study has several limitations. First, the small sample size resulted in limited power to detect differences between telomere length and COPD related phenotypes. Further large-scale studies with longer follow-up periods involving several serial assessments are needed to validate our findings.

Second, telomere length is a complex characteristic that is shaped by several factors, including genetic, epigenetic, lifestyle and environmental determinants. The complex

interactions of these factors remain unclear[33]. The association between telomere length and environmental, occupational, and medical risk factors has been reported in several cross-sectional epidemiological studies[34,35]. However, this study did not consider such environmental and occupational factors. And this study was consisted of the participants living in dusty areas near cement plants. This study results might differ from the general COPD populations. In CODA cohort study, we have some records of exposure such as air pollution (PM_{10}, NO_2). However, additional analysis could not be carried out due to insufficient number of participants.

Third, we measured the leukocyte telomere length. Regarding this point, it is important to note that a correlation between lung and blood telomere length has not been unequivocally demonstrated. This suggests that the associations between telomere length and various diseases cannot easily be interpreted as causative relations.

In conclusion, the correlation between lung function, respiratory symptoms, or extent and visual assessment and telomere length were analyzed, we did not find statistically significant results. Further studies are needed on the role of telomere length in COPD pathogenesis, as well as the relationship between telomere length and environmental factors including air pollution.

Authors' Contributions

Conceptualization: Kim WJ and Moon DH. Methodology: Kim WJ, Moon DH, and Kim JY. Formal analysis: Lim MN . Data Curation: Lim MN. Software: Lim MN. Validation: Lim MN, Kim WJ, and Moon DH. Investigation: Kim WJ, Moon DH, and Kim JY . Writing – original draft preparation: Moon DH and Kim WJ . Writing – review and editing: all authors . Approval of the final manuscript: All authors.

Conflicts of Interest

There is no potential conflict of interest relevant to this article was to disclose.

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Reference

1. Ito K, Barnes PJ. COPD as a disease of accelerated lung aging. *Chest.* 2009;135(1):173-180.
2. Vestbo J, Anderson W, Coxson HO, et al. Evaluation of COPD longitudinally to identify predictive surrogate end-points (ECLIPSE). *Eur Respir J.* 2008;31(4):869-873.
3. Blackburn EH. Structure and function of telomeres. *Nature.* 1991;350(6319):569-573.
4. Houben JM, Moonen HJ, van Schooten FJ, Hageman GJ. Telomere length assessment: biomarker of chronic oxidative stress? *Free Radic Biol Med.* 2008;44(3):235-246.
5. Lee J, Sandford AJ, Connett JE, et al. The Relationship between Telomere Length and Mortality in Chronic Obstructive Pulmonary Disease (COPD). *PLoS One.* 2012;7(4):e35567.
6. Rode L, Bojesen SE, Weischer M, Vestbo J, Nordestgaard BG. Short telomere length, lung function and chronic obstructive pulmonary disease in 46 396 individuals. *Thorax.* 2013;68(5):429-435.
7. 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;66(2):202-213.
8. Armanios M. Telomeres and age-related disease: how telomere biology informs clinical paradigms. *J Clin Invest.* 2013;123(3):996-1002.
9. Wu X, Amos CI, Zhu Y, et al. Telomere dysfunction: a potential cancer predisposition factor. *J Natl Cancer Ins.* 2003;95(16):1211-1218.
10. Calado RT, Young NS. Telomere diseases. *N Engl J Med.* 2009;361(24):2353-2365.
11. Serrano AL, Andrés V. Telomeres and cardiovascular disease: does size matter? *Circ Res.* 2004;94(5):575-584.
12. Silverman EK. Exacerbations in chronic obstructive pulmonary disease: do they contribute to disease progression? *Proc Am Thorac Soc.* 2007;4(8):586-590.
13. Han Y, Heo Y, Hong Y, Kwon SO, Kim WJ. Correlation between physical activity and lung function in dusty areas: results from the chronic obstructive pulmonary disease in dusty areas (CODA) cohort. *Tuberc Respir Dis.* 2019;82(4):311-318.
14. Mahler DA, Wells CK. Evaluation of clinical methods for rating dyspnea. *Chest.* 1988;93(3):580-586.
15. Celli BR, MacNee W, Agusti A, et al. Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. *Eur Respir J.* 2004;23(6):932-946.
16. Bak SH, Kwon SO, Han S-S, Kim WJ. Computed tomography-derived area and density of pectoralis muscle associated disease severity and longitudinal changes in chronic obstructive pulmonary disease: a case control study. *Respir Res.* 2019;20(1):226.
17. Washko GR, Hunninghake GM, Fernandez IE, et al. Lung volumes and emphysema in smokers with interstitial lung abnormalities. *N Engl J Med.* 2011;364(10):897-906.
18. Kim V, Davey A, Comellas AP, et al. Clinical and computed tomographic predictors of chronic bronchitis in COPD: a cross sectional analysis of the COPDGene study. *Respir Res.* 2014;15(1):1-9.
19. Lynch DA, Austin JH, Hogg JC, et al. CT-definable subtypes of chronic obstructive pulmonary disease: a statement of the Fleischner Society. *Radiology.* 2015;277(1):192-205.
20. Park J, Hobbs BD, Crapo JD, et al. Subtyping COPD by Using Visual and Quantitative CT Imaging Features. *Chest.* 2020;157(1):47-60.
21. O'Callaghan NJ, Fenech M. A quantitative PCR method for measuring absolute telomere length. *Biol Proced Online.* 2011;13(1):3.
22. Levy MZ, Allsopp RC, Futcher AB, Greider CW, Harley CB. Telomere end-replication

problem and cell aging. *J Mol Biol.* 1992;225(4):951-960.
23. Bernadotte A, Mikhelson VM, Spivak IM. Markers of cellular senescence. Telomere shortening as a marker of cellular senescence. *Aging (Albany NY).* 2016;8(1):3.
24. Clouston SA, Edelman NH, Aviv A, Stewart C, Luft BJ. Shortened leukocyte telomere length is associated with reduced pulmonary function and greater subsequent decline in function in a sample of World Trade Center responders. *Sci Rep.* 2019;9(1):1-7.
25. Albrecht E, Sillanpää E, Karrasch S, et al. Telomere length in circulating leukocytes is associated with lung function and disease. *Eur Respir J.* 2014;43(4):983-992.
26. Jin M, Lee EC, Ra SW, et al. Relationship of absolute telomere length with quality of life, exacerbations, and mortality in COPD. *Chest.* 2018;154(2):266-273.
27. Smith BM, Austin JH, Newell Jr JD, et al. Pulmonary emphysema subtypes on computed tomography: the MESA COPD study. *Am J Med.* 2014;127(1):94. e97-94. e23.
28. Dijkstra AE, Postma DS, ten Hacken N, et al. Low-dose CT measurements of airway dimensions and emphysema associated with airflow limitation in heavy smokers: a cross sectional study. *Respir Res.* 2013;14(1):11.
29. Karrasch S, Holz O, Jörres RA. Aging and induced senescence as factors in the pathogenesis of lung emphysema. *Respir Med.* 2008;102(9):1215-1230.
30. Babizhayev MA, Yegorov YE. Smoking and health: association between telomere length and factors impacting on human disease, quality of life and life span in a large population-based cohort under the effect of smoking duration. *Fundam Clin Pharmacol.* 2011;25(4):425-442.
31. Morla M, Busquets X, Pons J, Sauleda J, MacNee W, Agusti A. Telomere shortening in smokers with and without COPD. *Eur Respir J.* 2006;27(3):525-528.
32. Halper-Stromberg E, Cho MH, Wilson C, et al. Visual assessment of chest computed tomographic images is independently useful for genetic association analysis in studies of chronic obstructive pulmonary disease. *Ann Am Thorac Soc.* 2017;14(1):33-40.
33. Daniali L, Benetos A, Susser E, et al. Telomeres shorten at equivalent rates in somatic tissues of adults. *Nat Commun.* 2013;4(1):1-7.
34. Benetos A, Kark JD, Susser E, et al. Tracking and fixed ranking of leukocyte telomere length across the adult life course. *Aging cell.* 2013;12(4):615-621.
35. Starkweather AR, Alhaeeri AA, Montpetit A, et al. An integrative review of factors associated with telomere length and implications for biobehavioral research. *Nurs Res.* 2014;63(1):36.

Table 1. Baseline characteristics of the study participants

	All	COPD	Non-COPD	*p*-value
Participants, n (%)	446	285 (63.9)	161 (36.1)	
Telomere length	18.68±14.29	16.81±13.90	21.97±14.43	0.0002
Sex				<.0001
Male, n (%)	321 (72.0)	227 (79.7)	94 (58.4)	
Female, n (%)	125 (28.0)	58 (20.3)	67 (41.6)	
Age	72.54±7.09	72.86±7.01	71.96±7.22	0.199
Smoking				<.0001
Current, n (%)	95 (21.3)	74 (26.0)	21 (13.0)	
Former, n (%)	185 (41.5)	134 (47.0)	51 (31.7)	
None, n (%)	166 (37.2)	77 (27.0)	89 (55.3)	
Pack-year	17.40±23.36	20.22±25.08	12.26±18.98	0.001
CAT	16.20±9.63	17.11±9.60	14.60±9.50	0.008
mMRC	1.37±1.14	1.47±1.14	1.18±1.10	0.009
Pre-bronchodilator				
FVC L	2.86±0.80	2.89±0.82	2.79±0.75	0.195
FVC % predicted	93.46±19.99	92.28±20.86	95.57±18.21	0.095
FEV_1 L	1.86±0.59	1.74±0.58	2.08±0.54	<.0001
FEV_1 % predicted	83.88±23.29	76.19±21.07	97.48±20.73	<.0001
FEV_1/FVC	65.13±11.49	59.78±9.22	74.58±8.68	<.0001
Post-bronchodilator				
FVC L	2.99±0.80	3.10±0.81	2.81±0.75	0.001
FVC % predicted	97.74±19.21	98.55±19.36	96.32±18.92	0.239
FEV_1 L	1.94±0.59	1.83±0.57	2.14±0.56	<.0001
FEV_1 % predicted	87.47±22.63	80.10±19.99	100.50±21.15	<.0001
FEV_1/FVC	65.25±11.44	58.84±8.61	76.58±5.45	<.0001
IL-8 (n=359)	18.06±22.31	16.89±18.10	21.27±30.96	0.194
IL-6 (n=359)	2.52±3.48	2.51±3.66	2.57±2.94	0.867
CRP (n=359)	0.28±0.62	0.26±0.59	0.31±0.70	0.475

Data are presented as mean ± SD and analyzed with the t-test or Chi-square test.

COPD; chronic obstructive pulmonary disease, CRP; C-reactive protein, CAT , COPD Assessment Test ; MMRC, Modified Medical Research Council; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; IL-6, interleukin-6; IL-8 , interleukin-8

Table 2. Clinical demographic characteristics, respiratory symptom and lung function of all participants (n=446)

	All	Tertile 1	Tertile 2	Tertile 3	Unadjusted *P* Trend	Adjusted *P* Trend
		<8.54	8.54-23.54	>23.54		
Participants, n (%)	446	148(33.2)	149(33.4)	149(33.4)		
Telomere length	18.68±14.29	4.73±2.52	15.46±4.53	35.75±9.43	<.0001	
COPD					0.0007	
Yes, n(%)	285(63.9)	112(75.7)	91(61.1)	82(55.0)		
No, n(%)	161(36.1)	36(24.3)	58(38.9)	67(45.0)		
Sex					0.437	
Male, n (%)	321(72.0)	112(75.7)	106(71.1)	103(69.1)		
Female, n (%)	125(28.0)	36(24.3)	43(28.9)	46(30.9)		
Age	72.54±7.09	72.96±6.86	71.85±7.39	72.80±7.02	0.346	
Smoking					0.150	
Current, n (%)	95(21.3)	41(27.7)	26(17.5)	28(18.8)		
Former, n (%)	185(41.5)	61(41.2)	64(42.9)	60(40.3)		
None, n (%)	166(37.2)	46(31.1)	59(39.6)	61(40.9)		
Pack-year	17.40±23.36	21.28±25.45	14.05±18.87	16.97±24.82	0.029	
CAT	16.20±9.63	16.74±0.91	16.17±0.93	15.79±0.93	0.772	0.697
mMRC	1.37±1.14	1.31±0.11	1.38±0.11	1.33±0.11	0.863	0.855
Pre-bronchodilator						
FVC L	2.86±0.80	2.73±0.06	2.77±0.07	2.83±0.06	0.943	0.369
FVC % predicted*	93.46±19.99	94.16±1.79	93.53±1.84	97.07±1.82	0.176	0.249
FEV_1 L	1.86±0.59	1.76±0.05	1.82±0.05	1.88±0.05	0.410	0.143
FEV_1 %predicted*	83.88±23.29	83.50±2.02	83.78±2.08	88.66±2.05	0.034	0.077
FEV_1/FVC	65.13±11.49	64.19±1.00	66.03±1.03	66.90±1.02	0.031	0.096
Post-bronchodilator						
FVC L	2.99±0.80	2.89±0.06	2.87±0.06	2.97±0.06	0.793	0.284
FVC % predicted*	97.74±19.21	99.38±1.73	96.80±1.78	101.64±1.76	0.081	0.086
FEV_1 L	1.94±0.59	1.84±0.05	1.89±0.05	1.96±0.05	0.492	0.135
FEV_1 %predicted*	87.47±22.63	87.23±1.98	86.68±2.03	92.20±2.01	0.027	0.052
FEV_1/FVC	65.25±11.44	63.84±1.01	65.94±1.03	66.66±1.02	0.213	0.072
IL-8 (n=263)	16.89±18.10	16.82±17.86	15.73±13.28	18.37±22.92	0.664	
IL-6 (n=263)	2.51±3.66	2.56±4.18	2.71±3.49	2.19±2.95	0.668	
CRP (n=263)	0.26±0.59	0.27±0.58	0.29±0.73	0.22±0.41	0.740	
Exacerbation						
Moderate, n (%)	10 (3.5)	3 (2.7)	3 (3.3)	4 (4.9)	0.707	
Severe, n (%)	13 (4.6)	4 (3.6)	4 (4.4)	5 (6.1)	0.704	
Moderate or severe	18 (6.3)	6 (5.4)	6 (6.6)	6 (7.3)	0.850	

Study participants were divided into three groups based on telomere length, with the first tertile being the shortest and the third tertile being the longest.

Data are presented as mean ± SD and analyzed with the t-test or Chi-square test.

Adjusted variables : sex, age, smoking status, height
* Adjusted variables : sex, age, smoking status

COPD; chronic obstructive pulmonary disease, CRP; C-reactive protein, CAT , COPD Assessment Test ; MMRC, Modified Medical Research Council; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; IL-6, interleukin-6; IL-8 , interleukin-8

Table 3. Clinical demographic characteristics, respiratory symptom and pulmonary function of COPD patients (n=285)

		Tertile 1†	Tertile 2†	Tertile 3†	Unadjusted *P* trend	Adjusted *P* trend
		<7.39	7.39-20.58	>20.58		
Participants, n (%)	285	95(33.3)	95(33.3)	95(33.3)		
Telomere length	18.68±14.29	4.11±2.30	12.89±4.13	33.44±10.17	<.0001	
Sex					0.206	
Male, n (%)	227 (79.6)	79(83.2)	70(73.7)	78(82.1)		
Female, n (%)	58 (20.4)	16(16.8)	25(26.3)	17(17.9)		
Age	72.86±7.01	73.29±704	72.69±7.32	72.61±6.73	0.768	
Smoking					0.215	
Current, n (%)	74 (26.0)	28(29.5)	25(26.3)	21(22.1)		
Former, n (%)	134 (47.0)	48(50.5)	38(40.0)	48(50.5)		
None, n (%)	77 (27.0)	19(20.0)	32(33.7)	26(27.4)		
Pack-year	20.30±25.08	25.31±27.03	15.05±19.10	20.71±27.44	0.020	
CAT	17.11±9.60	16.87±1.18	17.57±1.21	16.67±1.27	0.680	0.886
mMRC	1.47±1.14	1.36±0.14	1.56±0.14	1.43±0.15	0.283	0.697
Pre-bronchodilator						
FVC L	2.89±0.82	2.77±0.08	2.88±0.09	2.89±0.09	0.823	0.217
FVC %predicted*	92.28±20.86	93.86±2.44	96.56±2.41	96.37±2.53	0.525	0.403
FEV_1 L	1.74±0.58	1.68±0.06	1.74±0.06	1.72±0.07	0.962	0.593
FEV_1 % predicted*	76.19±21.07	77.55±2.47	79.66±2.44	77.90±2.56	0.637	0.908
FEV_1/FVC	59.79±9.22	60.31±1.09	60.10±1.08	59.51±1.13	0.853	0.552
Post-bronchodilator						
FVC L	3.10±0.81	2.93±0.08	3.00±0.08	3.06±0.08	0.517	0.157
FVC % predicted*	98.55±19.36	99.22±2.28	100.67±2.25	102.23±2.37	0.562	0.319
FEV_1 L	1.83±0.57	1.76±0.06	1.79±0.06	1.81±0.06	0.734	0.512
FEV_1 % predicted*	80.10±19.99	81.25±2.35	82.12±2.32	81.66±2.45	0.881	0.889
FEV_1/FVC	58.84±8.61	59.46±1.02	59.33±1.01	58.97±1.06	0.931	0.695
IL-8 (n=263)	16.89±18.10	17.10±19.12	16.35±12.85	17.24±21.65	0.941	
IL-6 (n=263)	2.51±3.66	2.51±4.27	2.91±3.67	2.06±2.78	0.311	
CRP (n=263)	0.26±0.59	0.25±0.54	0.33±0.77	0.20±0.38	0.378	
Exacerbation						
Moderate, n (%)	10 (3.5)	3(3.2)	3(3.2)	4(4.2)	0.902	
Severe, n (%)	13 (4.6)	4(4.2)	4(4.2)	5(5.3)	0.923	
Moderate or severe, n (%)	18 (6.3)	6(6.3)	6(6.3)	6(6.3)	-	

† COPD patients were divided into three groups based on telomere length, with the first tertile being the shortest and the third tertile being the longest. Data are presented as mean ± SD and analyzed with the t-test or Chi-square test.

Adjusted variables : sex, age, smoking status, height
* Adjusted variables : sex, age, smoking status
COPD; chronic obstructive pulmonary disease, CRP; C-reactive protein, CAT , COPD Assessment Test ; MMRC, Modified Medical Research Council; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; IL-6, interleukin-6; IL-8 , interleukin-8

Table 4. Change in FVC and FEV_1 according to telomere length (All participants, n=446)

	Tertile 1	Tertile 2	Tertile 3	*P* trend
	<8.54	8.54-23.54	>23.54	
Pre-bronchodilator				
FVC (mL/yr)	-4.294±1.707	-5.086±1.898	-6.044±2.187	<.0001
FEV_1 (mL/yr)	-2.675±1.692	-3.796±1.820	-3.304±2.000	0.736
Post-bronchodilator				
FVC (mL/yr)	-2.467±1.577	-3.480±1.737	-2.578±1.990	0.952
FEV_1 (mL/yr)	-2.199±0.903	-2.709±0.975	-1.364±1.094	0.409

Study participants were divided into three groups based on telomere length, with the first tertile being the shortest and the third tertile being the longest.

Adjusted variables : sex, age, smoking status, height
FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 s

Table 5. Change in FVC and FEV_1 according to telomere length (COPD patients, n= 285)

	Tertile 1†	Tertile 2†	Tertile 3†	*P* trend
	<7.39	7.39-20.58	>20.58	
Pre-bronchodilator				
FVC (mL/yr)	-5.652±2.004	-6.358±2.248	-7.346±2.706	<.0001
FEV_1 (mL/yr)	-3.297±2.005	-5.225±2.197	-4.206±2.477	0.679
Post-bronchodilator				
FVC (mL/yr)	-5.003±1.900	-5.368±2.114	-4.606±2.518	0.856
FEV_1 (mL/yr)	-2.463±0.975	-2.958±1.078	-1.998±1.269	0.674

† COPD patients were divided into three groups based on telomere length, with the first tertile being the shortest and the third tertile being the longest.
Adjusted variables : sex, age, smoking status, height
FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 s

Table 6. The association between telomere length and visual and quantitative CT imaging features in all participants (n=421)

	All	All mean±SD	Tertile 1	Tertile 1 mean±SD	Tertile 2	Tertile 2 mean±SD	Tertile 3	Tertile 3 mean±SD	*P* Trend
			<8.54		8.54-23.54		>23.54		
Participants, n (%)	421		142 (33.7)		139 (33.0)		140 (33.3)		
Emphysema index	5.69±6.55		6.22±6.35		5.33±5.84		5.51±7.38		0.486
Mean Wall Area (%)	68.85±5.19		69.85±0.56		68.95±0.63		70.79±0.68		0.669
CT subtype									
Normal	199 (47.3)	19.02±1.27	55 (38.7)	4.31±0.96	67 (48.2)	15.20±0.92	77 (55.0)	34.67±0.87	
PSE	30 (7.1)	15.18±2.75	14 (9.9)	5.19±2.51	10 (7.2)	16.64±2.22	6 (4.3)	34.09±3.09	
Bronchial	10 (2.4)	24.11±4.47	0 (0.0)		5 (3.6)	15.90±5.21	5 (3.6)	33.78±5.93	
Trace	48 (11.4)	14.94±2.17	23 (16.2)	2.10±1.58	12 (8.6)	15.22±1.86	13 (9.3)	34.43±1.93	
Mild	82 (19.5)	16.90±1.75	29 (20.4)	4.63±1.48	32 (23.0)	12.81±1.62	21 (15.0)	35.33±1.74	
Moderate	38 (9.0)	18.01±2.44	17 (12.0)	4.61±2.95	11 (7.9)	16.42±3.77	10 (7.1)	38.50±3.75	
Confluent and advanced	14 (3.3)	23.90±3.94	4 (2.8)	0.64±5.32	2 (1.4)	11.38±5.78	8 (5.7)	35.47±3.14	
***P* Trend**		0.179		0.537		0.518		0.830	

Study participants were divided into three groups based on telomere length, with the first tertile being the shortest and the third tertile being the longest.

Adjusted variables: sex, age, smoking status

CT, computed tomography; SD, standard deviation; PSE, paraseptal emphysema

Table 7. The OR of Exacerbation according to telomere length (COPD patients , n= 285)

	Moderate	Severe	Moderate or severe
Telomere length			
tertile 1† vs tertile 3†	**0.707(0.153-3.274)**	**0.788(0.203-3.057)**	**0.997(0.307-3.241)**
tertile 2† vs tertile 3†	**0.681(0.146-3.184)**	**0.781(0.200-3.050)**	**0.966(0.295-3.157)**
***p*-value**	**0.859**	**0.919**	**0.998**

† COPD patients were divided into three groups based on telomere length, with the first tertile being the shortest and the third tertile being the longest.

Adjusted variables : sex, age, smoking status, height

Supplement

Table 1. Clinical demographic characteristics, respiratory symptom and pulmonary function of non-COPD patients (n=161)

		Tertile 1‡	**Tertile 2**‡	**Tertile 3**‡	**Unadjusted *P* Trend**	**Adjusted *P* Trend**
		<12.22	12.22-27.91	>27.91		
Participants, n (%)	161	53(33.0)	54(33.5)	54(33.5)		
Telomere length	21.97±14.43	6.61±0.35	20.07±4.21	38.95±8.12	<.0001	
Sex					0.429	
Male, n (%)	94(58.4)	34(64.2)	32(59.3)	28(51.9)		
Female, n (%)	67(41.6)	19(35.8)	22(40.7)	26(48.1)		
Age	71.96±7.22	71.82±7.30	70.94±7.19	73.13±7.14	0.287	
Smoking					0.088	
Current, n (%)	21(13.0)	11(20.8)	5(9.3)	5(9.3)		
Former, n (%)	51(31.7)	14(26.4)	23(42.6)	14(25.9)		
None, n (%)	89(55.3)	28(52.8)	26(48.1)	35(64.8)		
Pack-year	12.26±18.98	12.85±18.70	14.16±20.75	9.78±17.41	0.472	
CAT	14.59±9.50	15.54±1.55	15.02±1.50	13.77±1.57	0.605	0.349
mMRC	1.18±1.10	1.11±0.18	1.30±0.17	1.11±0.18	0.729	0.994
Pre-bronchodilator						
FVC L	2.79±0.75	2.64±0.08	2.63±0.08	2.74±0.08	0.989	0.317
FVC % predicted*	95.56±18.21	92.48±2.76	91.03±2.71	97.20±2.83	0.074	0.167
FEV_1 L	2.08±0.54	1.97±0.07	2.00±0.06	2.11±0.07	0.768	0.097
FEV_1 % predicted*	97.48±20.73	94.53±3.08	94.13±3.02	102.25±3.16	0.022	0.044
FEV_1/FVC	74.58±8.68	73.54±1.30	76.78±1.27	77.05±1.33	0.044	**0.030**
Post-bronchodilator						
FVC L	2.81±0.75	2.75±0.09	2.65±0.09	2.81±0.09	0.864	0.595
FVC % predicted*	96.32±18.92	95.83±2.84	92.14±2.79	99.30±2.92	0.086	0.324
FEV_1 L	2.14±0.56	2.07±0.07	2.05±0.07	2.19±0.07	0.809	0.150
FEV_1 % predicted*	100.51±21.15	98.69±3.17	96.56±3.11	106.30±3.25	0.017	0.053

FEV_1/FVC	76.58±5.45	75.52±0.84	77.7±0.82	78.48±0.86	0.011	**0.005**
IL-8 (n=96)	21.27±30.96	26.06±42.41	17.09±13.09	17.73±19.68	0.396	
IL-6 (n=96)	2.57±2.94	3.03±3.23	2.08±2.69	2.33±2.69	0.376	
CRP (n=96)	0.31±0.70	0.40±0.81	0.30±0.79	0.18±0.21	0.468	

‡ Non COPD patients were divided into three groups based on telomere length, with the first tertile being the shortest and the third tertile being the longest.
Data are presented as mean ± SD and analyzed with the t-test or Chi-square test.

Adjusted variables : sex, age, smoking status, height
* Adjusted variables : sex, age, smoking status

COPD; chronic obstructive pulmonary disease, CRP; C-reactive protein, CAT , COPD Assessment Test ; MMRC, Modified Medical Research Council; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; IL-6, interleukin-6; IL-8 , interleukin-8

Supplement

Table 2. Change in FVC and FEV_1 according to telomere length (non-COPD patients, n= 161)

	Tertile 1‡	**Tertile 2‡**	**Tertile 3‡**	***P* trend**
	<12.222	12.222-27.912	>27.912	
Pre-bronchodilator				
FVC (mL/yr)	-1.847±3.794	0.169±3.692	-6.717±4.450	0.148
FEV_1 (mL/yr)	2.781±3.974	0.397±4.134	1.818±4.695	0.809
Post-bronchodilator				
FVC (mL/yr)	0.138±3.053	2.861±2.932	3.399±3.655	<.0001
FEV_1 (mL/yr)	1.703±2.344	0.839±2.323	2.764±2.758	0.633

‡ Non COPD patients were divided into three groups based on telomere length, with the first tertile being the shortest and the third tertile being the longest.
Adjusted variables : sex, age, smoking status, height
FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 s

Supplement

Table 3. The association between telomere length and visual and quantitative CT imaging features in COPD patients (n=272)

		All mean±SD	Tertile 1†	Tertile 1† mean±SD	Tertile 2†	Tertile 2† mean±SD	Tertile 3†	Tertile 3† mean±SD	*P* Trend
			<7.39		7.39-20.58		>20.58		
Participants, n (%)	272		92(33.8)		87(32.0)		93(34.2)		
Emphysema index	7.76±7.40		7.09±0.85		6.48±0.85		6.47±0.90		0.553
Mean Wall Area (%)	69.36±4.92		70.07±0.58		69.67±0.58		70.77±0.61		0.328
CT subtype									
Normal	89(32.7)	18.00±13.32	25(27.2)	4.24±0.57	29(33.3)	12.46±0.96	35(37.6)	31.35±2.31	
PSE	27(9.9)	14.02±12.51	13(14.1)	4.64±0.75	8(9.2)	14.60±1.66	6(6.5)	32.22±4.65	
Bronchial	6(2.2)	22.88±12.18	0(0.0)	-	3(3.5)	12.89±2.45	3(3.2)	32.22±6.21	
Trace	35(12.9)	14.44±13.68	17(18.5)	3.63±0.67	9(10.3)	15.21±1.48	9(9.7)	32.82±3.85	
Mild	68(25.0)	16.88±14.29	19(20.6)	4.07±0.60	28(32.2)	11.93±0.98	21(22.6)	33.95±2.75	
Moderate	33(12.1)	16.18±16.03	14(15.2)	3.79±0.71	8(9.2)	11.59±1.61	11(11.8)	33.98±3.61	
Confluent and advanced	14(5.2)	22.26±16.20	4(4.4)	3.14±1.23	2(2.3)	11.59±3.01	8(8.6)	33.20±4.10	
***P* Trend**		0.390		0.822		0.407		0.992	

† COPD patients were divided into three groups based on telomere length, with the first tertile being the shortest and the third tertile being the longest.
Adjusted variables: sex, age, smoking status

CT, computed tomography; SD, standard deviation; PSE, paraseptal emphysema

Supplement

Table 4 . The association between telomere length and visual and quantitative CT imaging features in non COPD participants (n=149)

		All mean±SD	Tertile 1‡	Tertile 1‡ mean±SD	Tertile 2‡	Tertile 2‡ mean±SD	Tertile 3‡	Tertile 3‡ mean±SD	*P* Trend
			<12.22		12.22-27.91		>27.91		
Participants, n (%)	149		50(33.6)		51(34.2)		48(32.2)		
Emphysema index	3.31±3.60		2.73±0.59		3.54±0.63		2.46±0.75		0.744
Mean Wall Area (%)	67.48±5.52		68.03±098		67.63±1.05		66.88±1.25		0.415
CT subtype									
Normal	110(73.8)	22.65±14.55	35(70.0)	6.91±0.69	36(70.6)	21.24±1.11	39(81.2)	36.79±2.02	
PSE	3(2.0)	18.75±5.69	0(0.0)	-	3(5.9)	19.69±2.71	0(0.0)	-	
Bronchial	4(2.7)	27.79±9.09	0(0.0)	-	2(3.9)	19.82±3.07	2(4.2)	35.33±6.15	
Trace	13(8.7)	20.03±17.63	6(12.0)	3.96±1.39	3(5.9)	23.18±2.59	4(8.3)	38.07±4.55	
Mild	14(9.4)	13.88±9.98	7(14.0)	6.30±1.34	5(9.8)	16.02±2.00	2(4.2)	37.45±6.45	
Moderate	5(3.4)	18.91±12.97	2(4.0)	7.31±2.45	2(3.9)	18.96±3.09	1(2.1)	42.08±8.77	
Confluent and advanced	0(0.0)	-	0(0.0)	-	0(0.0)	-	0(0.0)	-	
***P* Trend**		0.556		0.241		0.225		0.970	

‡ Non COPD patients were divided into three groups based on telomere length, with the first tertile being the shortest and the third tertile being the longest.
Adjusted variables: sex, age, smoking status

CT, computed tomography; SD, standard deviation; PSE, paraseptal emphysema