Short telomere length, lung function and chronic obstructive pulmonary disease in 46 396 individuals

Line Rode,1 Stig E Bojesen,1,2,3 Maren Weischer,1,3 Jørgen Vestbo,4,5 Børge G Nordestgaard1,2,3

ABSTRACT

Background A previous case–control study of 100 individuals found that short telomere length was associated with a 28-fold increased risk of chronic obstructive pulmonary disease (COPD).

Objectives To test the hypothesis that short telomere length is associated with reduced lung function and an increased risk of COPD.

Methods Observational study of 46 396 individuals from the Danish general population.

Measurements Leucocyte telomere length and spirometry were measured. COPD was defined using either forced expiratory volume in 1 s (FEV1)/forced vital capacity (FVC) ratio <0.70 as suggested by the Global Initiative for chronic Obstructive Lung Disease (GOLD) or FEV1/FVC below the lower limit of normal (LLN).

Results Telomere length decreased significantly with increasing age (p=10−300). FEV1, FVC and FEV1/FVC decreased with decreasing telomere length quartiles (p trend: 5×10−51, 5×10−35 and 6×10−117, respectively), but the associations attenuated after age and multivariable adjustment. The risk of COPD increased with decreasing telomere length quartile (p trend: 5×10−51, 5×10−35 and 6×10−117, respectively), but the associations attenuated after age and multivariable adjustment. Unadjusted and multivariable adjusted OR for shortest versus longest telomere length quartiles were 2.06 (95% CI 1.91 to 2.22) and 1.15 (95% CI 1.06 to 1.25) for GOLD and 1.73 (95% CI 1.60 to 1.88) and 1.19 (95% CI 1.09 to 1.30) for FEV1/FVC below LLN, respectively. Per 1000 base pairs decrease in telomere length, the multivariable adjusted OR was 1.07 (95% CI 1.03 to 1.10) for GOLD and 1.07 (95% CI 1.03 to 1.11) for FEV1/FVC below LLN.

Conclusions Short telomere length is associated with decreased lung function and with increased risk of COPD, but the associations are markedly attenuated after adjustment. Our data support a modest correlation between telomere length and the lung function indices examined.

INTRODUCTION

Lung tissue is often exposed to oxidants from inhaled nitrogen dioxide, ozone, gasoline and diesel exhaust and from tobacco smoke. The combination of a large surface area and a high blood supply result in a high susceptibility to oxidative stress in lung tissue,1 a factor that is believed to play a crucial role in pulmonary inflammation, decreased pulmonary function and in the development of chronic obstructive pulmonary disease (COPD).

Telomeres are repetitive DNA sequences at the end of chromosomes which are important for chromosome protection and thus for longevity of cells.2 3 In most cells telomere length shortens with each cell division, and since exposure to oxidative stress as well as inflammation enhance this shortening, telomere length may serve as a marker for cellular ageing.4 5 In accordance with this notion, telomere length decreases with increasing age, and short telomere length is also associated with lifestyle factors such as smoking, obesity and stress.6–9 It is therefore plausible that pulmonary oxidative stress and inflammation could lead to shorter telomere length, and that short telomere length could be a marker of decreased lung function and increased risk of COPD.
We tested the hypothesis that short telomere length is associated with decreased lung function and increased risk of COPD in a sample of 46,396 participants from the Danish general population. Severity of COPD was defined according to forced expiratory volume in 1 s (FEV$_1$) and forced vital capacity (FVC), either by the Global initiative for chronic Obstructive Lung Disease (GOLD) criteria or by FEV$_1$/FVC below the lower limit of normal (LLN).\textsuperscript{10, 11}

**METHODS**

**Population**

We studied participants from the Copenhagen General Population Study and the Copenhagen City Heart Study, both of which are Danish prospective general population studies.\textsuperscript{12-14} In both studies, randomly selected Copenhagen residents were invited to complete a questionnaire and undergo a physical examination including spirometry. Whole blood samples were collected for DNA isolation. There was no overlap of individuals between the two studies.

The study population consisted of 37,355 participants from the Copenhagen General Population Study included between 2003 and 2007 and 9041 participants from the Copenhagen City Heart Study included between 1991 and 1994, all with available spirometry and telomere length measurement. A total of 16,563 individuals were invited to participate in The Copenhagen City Heart Study between 1991 and 1994 and of these 10,135 entered the study. Whole blood samples for DNA isolation were collected in 9,252 individuals, and among these 9,041 had information on telomere length and spirometry. The Copenhagen General Population Study is an ongoing study. A total of 46% of invited individuals participated and 99% had whole blood samples collected for DNA isolation. Spirometry data are available for 98% of participants.

**Lung function and COPD**

At each examination three sets of values were registered for FEV$_1$ and FVC and the highest set of values was used in the analyses. At least two measurements of FEV$_1$ and FVC were used on a continuous scale in other analyses. For logistic regression analysis, C-reactive protein levels were categorised according to lowest, middle and highest tertiles but used on a continuous scale in other analyses.

**Statistical analyses**

The association between telomere length continuously with age and between telomere length quartiles and lung function was assessed by general linear models while the association with COPD was assessed by logistic regression. Multivariable models were adjusted for age, gender, body mass index, smoking status, smoking inhalation, cumulative smoking in pack-years, occupational exposure to dust and fumes (yes/no) and dyspnoea. Participants were classified as never smokers, former smokers or current smokers. For former smokers and current smokers, pack-years of cigarettes or equivalent smoking were calculated and information on inhalation was noted as yes/no. One pack-year corresponds to one pack of 20 cigarettes or equivalent smoked for a year. The severity of dyspnoea was graded from 0 to 4 according to the modified Medical Research Council Dyspnoea Scale.\textsuperscript{20} Questionnaires were completed and reviewed by an examiner on the day of examination. Height and weight were registered on the day of examination. Body mass index was calculated as the measured weight divided by the square of the measured height and categorised according to the WHO classification into body mass index $<$18.5 kg/m$^2$, 18.5–24.9 kg/m$^2$, 25–29.9 kg/m$^2$ and $\geq$30 kg/m$^2$. The number of years in school were categorised as $<$8 years, 8–11 years and $>$11 years. For logistic regression analysis, C-reactive protein levels were categorised according to lowest, middle and highest tertiles but used on a continuous scale in other analyses.

**RESULTS**

Figure 1 shows the decrease in telomere length on a continuous scale with increasing age in the 46,396 participants from the general population ($p$$<$1×10$^{-300}$). Decreasing telomere length in quartiles was associated with increased age, male gender, and current smoking. Smoking inhalation was used on a continuous scale in other analyses.
increased body mass index, former smoking status, smoking inhalation, increased cumulative smoking, increased occupational exposure to dust and fumes, <8 years in school and with increased C-reactive protein levels (table 1). Similar patterns were seen when analysing telomere length as a continuous variable (ie, change in lung function per 1000 base pair decrease in telomere length; figure 3). Stratified analyses of the change in unadjusted FEV1/FVC per 1000 base pair decrease in telomere length showed a decrease in all strata (figure 4). FEV1/FVC decreased slightly more in women than in men, in individuals aged ≥60 years than in those aged <60 years of age and in former and current smokers than in never smokers.

A total of 6770 participants (15%) had an FEV1/FVC < 0.70. Among these, 2922 (43%) were classified as GOLD stage I, 3026 (45%) as GOLD stage II and 822 (12%) as GOLD stages III-IV. A total of 5136 participants (11%) met the criteria for COPD by FEV1/FVC below LLN. Table S1 in the online supplement shows the baseline characteristics of participants according to these COPD criteria.

Figure 5 shows the unadjusted, age adjusted and multivariable adjusted logistic regression analyses of the association between decreasing telomere length in quartiles and COPD risk according to the GOLD criteria and FEV1/FVC below LLN. In the unadjusted analyses, the OR of COPD increased with decreasing telomere length quartiles (p trend: p = 7×10^{-92} for GOLD criteria; p = 8×10^{-94} for FEV1/FVC below LLN). In the

### Table 1 Baseline characteristics of participants by quartiles of telomere length

<table>
<thead>
<tr>
<th>Telomere length, base pairs</th>
<th>1st quartile (longest)</th>
<th>2nd quartile</th>
<th>3rd quartile</th>
<th>4th quartile (shortest)</th>
<th>p For trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>11599</td>
<td>11600</td>
<td>11599</td>
<td>11598</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>53 (43–62)</td>
<td>56 (46–65)</td>
<td>60 (50–69)</td>
<td>65 (56–73)</td>
<td>&lt;1×10^{-100}</td>
</tr>
<tr>
<td>Men, n (%)</td>
<td>5026 (43)</td>
<td>5155 (44)</td>
<td>5433 (47)</td>
<td>5740 (49)</td>
<td>1×10^{-22}</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25 (23–28)</td>
<td>26 (23–28)</td>
<td>26 (23–29)</td>
<td>26 (23–29)</td>
<td>5×10^{-48}</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Former smoking, n (%)</td>
<td>4236 (37)</td>
<td>4512 (39)</td>
<td>4787 (41)</td>
<td>5121 (44)</td>
<td>3×10^{-33}</td>
</tr>
<tr>
<td>Current smoking, n (%)</td>
<td>2857 (25)</td>
<td>2758 (24)</td>
<td>2909 (25)</td>
<td>2841 (25)</td>
<td>0.14</td>
</tr>
<tr>
<td>Smoking inhalation, n (%)</td>
<td>5927 (84)</td>
<td>5909 (81)</td>
<td>6032 (78)</td>
<td>5984 (75)</td>
<td>1×10^{-39}</td>
</tr>
<tr>
<td>Cumulative smoking, pack-years</td>
<td>5 (0–22)</td>
<td>6 (0–24)</td>
<td>9 (0–29)</td>
<td>12 (0–32)</td>
<td>4×10^{-103}</td>
</tr>
<tr>
<td>Occupational exposure to dust/fumes, n (%)</td>
<td>1499 (13)</td>
<td>1531 (13)</td>
<td>1650 (14)</td>
<td>1766 (15)</td>
<td>4×10^{-7}</td>
</tr>
<tr>
<td>&lt;8 years in school, n (%)</td>
<td>1635 (14)</td>
<td>1820 (16)</td>
<td>2289 (20)</td>
<td>2754 (24)</td>
<td>4×10^{-94}</td>
</tr>
<tr>
<td>C-reactive protein, mg/l</td>
<td>1.4 (0.8–2.6)</td>
<td>1.5 (1.0–2.6)</td>
<td>1.6 (1.1–2.8)</td>
<td>1.7 (1.2–3.1)</td>
<td>5×10^{-152}</td>
</tr>
</tbody>
</table>

Continuous variables are shown as medians (IQR) and categorical variables are shown as numbers (%).
unadjusted analyses, OR for the shortest versus the longest telo-
mere quartiles was 2.06 (95% CI 1.91 to 2.22) for GOLD cri-
teria and 1.73 (1.60 to 1.88) for FEV1/FVC below LLN. The
association was markedly attenuated when analyses were
adjusted for age or multivariable adjusted for age, gender, body
mass index, smoking status, smoking inhalation, cumulative
smoking, occupational exposure to dust and fumes, years in
school, C-reactive protein levels, grade of dyspnoea and study.
In the multivariable models the OR for the shortest versus
longest telomere quartiles was 1.15 (95% CI 1.06 to 1.25) for
GOLD criteria and 1.19 (95% CI 1.09 to 1.30) for FEV1/FVC
below LLN. Trend tests across quartiles after multivariable
adjustment yielded p values of 0.001 and 6×10−5, respectively,
for GOLD criteria and FEV1/FVC below LLN.

Per 1000 base pairs decrease in telomere length, the multi-
variable adjusted OR was 1.07 (95% CI 1.03 to 1.10) for
GOLD criteria and 1.07 (1.03 to 1.11) for FEV1/FVC below
LLN (figure 6). We stratified according to gender, age, smoking
status and cumulative smoking in multivariable adjusted models
and did not find evidence of an interaction between age,
smoking status or cumulative smoking and decreasing telomere
length on risk of COPD classified as GOLD or as FEV1/FVC
below LLN. For GOLD we found a p value for test of inter-
action of 0.01 for men compared with women (required p value
after Bonferroni correction <0.05/4=0.013). However, the cor-
responding p value for FEV1/FVC below LLN was 0.75.

The results from the two studies separately are shown in
tables S2–S5 and figures S1–S4 in the online supplement. The

Table 2 Association between telomere length and covariables individually by univariable regression analysis and in combination by
multivariable regression analysis

<table>
<thead>
<tr>
<th></th>
<th>Univariable</th>
<th></th>
<th>Multivariable</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient (95% CI)</td>
<td>p Value</td>
<td>Coefficient (95% CI)</td>
<td>p Value</td>
</tr>
<tr>
<td>Age, years</td>
<td>−22.1 (−22.7 to −21.4)</td>
<td>&lt;1×10−300</td>
<td>−21.5 (−22.2 to −20.8)</td>
<td>&lt;1×10−300</td>
</tr>
<tr>
<td>Male gender</td>
<td>−102.1 (−120.1 to −84.1)</td>
<td>1×10−28</td>
<td>−88.8 (−106.0 to −71.6)</td>
<td>4×10−24</td>
</tr>
<tr>
<td>Cumulative smoking in pack-years</td>
<td>−5.2 (−5.6 to −4.8)</td>
<td>4×10−126</td>
<td>−1.4 (−1.9 to −0.9)</td>
<td>3×10−8</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>−13.2 (−15.3 to −11.1)</td>
<td>2×10−13</td>
<td>−5.3 (−7.3 to −3.3)</td>
<td>3×10−7</td>
</tr>
<tr>
<td>Smoking inhalation</td>
<td>−17.2 (−35.3 to −0.8)</td>
<td>0.04</td>
<td>−39.3 (−58.8 to −19.9)</td>
<td>8×10−5</td>
</tr>
<tr>
<td>&lt;8 years in school</td>
<td>−250.7 (−274.3 to −227.1)</td>
<td>2×10−36</td>
<td>−25.9 (−39.3 to −12.4)</td>
<td>2×10−4</td>
</tr>
<tr>
<td>C-reactive protein, mg/l</td>
<td>−8.4 (−10.1 to −6.8)</td>
<td>4×10−23</td>
<td>−2.8 (−4.3 to −1.2)</td>
<td>4×10−4</td>
</tr>
<tr>
<td>Ever smoking</td>
<td>−130.2 (−140.1 to −111.2)</td>
<td>1×10−43</td>
<td>Omitted*</td>
<td>–</td>
</tr>
<tr>
<td>Exposure to dust or fumes</td>
<td>−66.5 (−92.5 to −40.4)</td>
<td>2×10−7</td>
<td>Omitted*</td>
<td>–</td>
</tr>
</tbody>
</table>

*Omitted by backward elimination of variables (for p<0.1) in stepwise linear regression analysis.

Figure 2 Forced expiratory volume in 1 s (FEV1) % predicted, forced vital capacity (FVC) % predicted and FEV1/FVC by quartiles of telomere
length. Values are shown as mean and SE. Multivariable adjustments include age, gender, body mass index, smoking status, smoking inhalation, cumulative
smoking in pack-years, occupational exposure to dust and fumes, years in school, C-reactive protein levels, grade of dyspnoea and study.
two studies showed similar results for all analyses. We repeated all analyses excluding individuals where a telomere length measurement had been obtained after a rerun of the blood sample. These sensitivity analyses did not change any of the main results or conclusions (data not shown).

**DISCUSSION**

The most important and novel finding from this observational study of a large sample of the Danish general population is that, although short telomere length is associated with reduced lung function and with an increased risk of COPD, these associations are attenuated markedly after age and multivariable adjustment. However, even after multivariable adjustment, a decrease in telomere length of 1000 base pairs was associated with a 1.07-fold risk of COPD by GOLD criteria as well as by FEV₁/FVC below LLN. Nevertheless, we cannot totally exclude that this remaining association could simply be a result of residual confounding. These results demonstrate an effect, albeit small, of an independent association between reduced telomere length and the risk of lung disease. It could be argued that telomere length is a marker of premature ageing and, as such, a mechanism by which other factors such as age, gender and smoking act. Therefore, telomere length could remain of interest even if not independently associated with lung function or risk of COPD.

The biological mechanism behind the finding of an association between decreased telomere length and decreased lung function and increased risk of COPD could be accelerated cell turnover in individuals with COPD, caused by inflammation and oxidative stress induced by inhalation of factors such as nitrogen dioxide, ozone, gasoline and diesel exhaust and tobacco smoke. Another hypothesis is that ageing and exposure to cigarette smoke entail lymphocyte senescence which increase susceptibility to infection and eventually lead to the chronic inflammation characteristic of COPD. 21 COPD may also be associated with a genetic predisposition to shorter telomere length due to decreased telomerase activity, a theory supported by findings of shorter telomere length and lower telomerase activity in cultured pulmonary cells from patients with COPD.
compared with control subjects. Also, in a recent study Alder et al. examined telomerase null mice with short telomeres and found that short telomere length could be a sign of genetic disposition to emphysema by increasing susceptibility to cigarette smoke-induced lung damage. Taken together, it is biologically plausible that short telomere length is associated with reduced lung function and an increased risk of COPD; however, the precise mechanism is unknown.

Our results are supported by previous findings of an association between telomere length and lung function and risk of COPD. Published studies have mainly been case-control studies of 26-283 COPD cases and 20-155 control subjects. In our cohort of 6770 individuals with COPD we found a 1.15-fold increased risk of COPD for individuals with the shortest telomere lengths compared with those with the longest telomere lengths. To contrast this with previous findings, Savale et al. compared 50 COPD patients with 50 matched control subjects and found a 28-fold increased risk of COPD for individuals with short telomere length compared with individuals with long telomere length. Two other small case-control studies were unable to show an association between telomere length and lung function but found an association between smoking exposure and telomere length. Mui et al. also demonstrated an association between telomere length and lung function, but this study included COPD cases only. Overall, the results of previous studies support the notion that short telomere length is associated with decreased lung function and hence the risk of COPD. The present study of a very large

Figure 5  Logistic regression analysis of risk of chronic obstructive pulmonary disease (COPD) according to telomere length in quartiles. Multivariable adjustments include age, gender, body mass index, smoking status, smoking inhalation, cumulative smoking in pack-years, occupational exposure to dust and fumes, years in school, plasma C-reactive protein levels, grade of dyspnoea and study. COPD was defined either by the Global initiative for chronic Obstructive Lung Disease (GOLD) criteria or by forced expiratory volume in 1 s/forced vital capacity ratio (FEV1/FVC) below lower limit of normal.

Figure 6  Risk of being classified as chronic obstructive pulmonary disease (COPD) by the Global initiative for chronic Obstructive Lung Disease (GOLD) criteria or by forced expiratory volume in 1 s/forced vital capacity ratio (FEV1/FVC) below lower limit of normal (LLN) per 1000 base pair decrease in telomere length. Multivariable adjustments include age, gender, body mass index, smoking status, smoking inhalation, cumulative smoking in pack-years, occupational exposure to dust and fumes, years in school, plasma C-reactive protein levels, grade of dyspnoea and study. COPD was defined either by the GOLD criteria or by FEV1/FVC ratio below LLN.
sample from a general population suggests that the association is more moderate than previously reported in smaller case-control studies.

A strength of our study is the large cohort with telomere length measurement and spirometry data from 46,396 individuals from the Danish general population. Also, importantly, we used two different classifications of COPD, examining 6,770 individuals classified as COPD by GOLD criteria and 5,136 by FEV1/FVC below LLN, and found similar results for the two COPD classifications. In addition, we combined data from two independent studies to obtain additional power, but the fact that these studies showed comparable results individually for the association between short telomeres and decreased lung function and increased risk of COPD adds additional credibility to the present study. Finally, the very strong association between decreasing telomere length and increasing age demonstrates the quality of our telomere length measurement.

One limitation of our study is that we cannot exclude the presence of residual confounding, despite the fact that we have included potential confounders such as age, gender, body mass index, smoking status, smoking inhalation, cumulative smoking, occupational exposure to dust and fumes, educational level, C-reactive protein levels and dyspnoea. Another limitation is that we did not have information on 6-min walking distance and therefore were not able to adjust for the entire Body mass index, airway Obstruction, Dyspnoea, and Exercise capacity (BODE) Index. Nevertheless, we did adjust for the three most important factors—namely, lung function, body mass index and grade of dyspnoea. The finding that the association was only moderate could also be explained by an underrepresentation of the most severe cases of COPD in our population, which may have underestimated the association between short telomere length and risk of COPD in this present study. Finally, as we studied white subjects only, our results may not necessarily apply to other races.

In conclusion, short telomere length is associated with decreased lung function and with increased risk of COPD, according to the GOLD classification as well as according to FEV1/FVC below LLN. However, the association seems to be only moderate and our results stress the importance of adjusting for age and other confounders when examining the association between COPD and short telomeres. Whether telomere shortening per se increases risk of COPD is still an unresolved question and should be examined in a study designed to evaluate the causal direction of the association.

Acknowledgements We thank laboratory technician Anja Johcusen for assisting with the telomere measurements. The authors are indebted to the staff and participants in the Copenhagen General Population Study and the Copenhagen City Heart Study.

Contributors LR, SEB and BGN initiated the study. LR performed statistical analyses supervised by SEB, JV and BGN. All authors analysed and interpreted the results. BGN, MW and SEB collected data. LR drafted the manuscript which was scrutinised by the other authors, all of whom accepted the final submitted manuscript.

Funding This study was financially supported by Chief Physician Johan Boserup and Lise Boserup's Foundation, the Copenhagen County Foundation, Herlev Hospital, Copenhagen University Hospital and the Danish Heart Foundation.

Competing interests None.

Patient consent Written informed consent was obtained from all participants.

Ethics approval Ethical approval was obtained from Herlev Hospital and Danish ethics committees (H-K01-144/01 and KF100.2039/91).

Provenance and peer review Not commissioned; internally peer reviewed.

REFERENCES