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Title: Genetic and Environmental Effects on Telomere Length and Lung Function : A Twin Study

Year: 2017

Version:

Please cite the original version:

Sillanpää, E., Sipilä, S., Törmäkangas, T., Kaprio, J., & Rantanen, T. (2017). Genetic and Environmental Effects on Telomere Length and Lung Function : A Twin Study. Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 72(11), 1561-1568. https://doi.org/10.1093/gerona/glw178

Genetic and environmental effects on telomere length and lung function: a twin study

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Running title: Twin study: telomeres and lung function

ABSTRACT

Background: The purpose of the study was to estimate the heritability of leucocyte telomere length (LTL) and lung function and to examine whether LTL and lung function share genetic or environmental effects in common.

Methods: 386 monozygotic (MZ) and dizygotic (DZ) Finnish twin sisters (age 68.4±3.4 years) were included. Relative LTL was determined from peripheral blood DNA by qPCR. Lung function measures of FEV_1, FVC, FEV_1/FVC and PEF were derived from spirometry. Genetic modeling was performed with MPlus statistical software.

Results: Univariate analysis revealed that in LTL 62% (95% CI 50-72) of the variance was explained by additive genetic and 38% (28-50) by unique environmental factors. For FEV_1, FVC and PEF the corresponding estimates were 65-67% for additive genetic and 33-35% for unique environmental factors. Across the sample, the phenotypic correlation between LTL and FEV_1 was modest (r=0.104, p=0.041). Bivariate correlated factors model revealed that the genetic correlation between LTL and FEV_1 was 0.18 (-0.19 to 0.64) and environmental correlation -0.10 (-0.84 to 0.55).

Conclusions: Both LTL and lung function variables are moderately to highly genetically determined. The associations between LTL and the lung function variables were weak. However, the positive genetic correlation point estimate gave minor suggestions that, in a larger sample, genetic factors in common might play a role in the phenotypic correlation between LTL and FEV_1. Future studies with larger samples are needed to confirm these preliminary findings.

Keywords: telomeres, spirometry, genetic modeling, discordant twins

INTRODUCTION

Telomeres are specialized nucleoprotein structures located at the end of chromosomes that act as protective caps contributing to genomic integrity and stability. In most human cells, telomeres shorten with every cell division and therefore telomere length has been hypothesized to be able to serve as a marker of biological aging (1). Telomere length reflects the length of the telomere at birth and its rate of attrition thereafter.

To a large extent, leucocyte telomere length (LTL) is genetically determined in children and adults, but it has also been reported that environmental factors may have a primary role on LTL maintenance in older people (2). LTL has been shown to be associated with mortality (3, 4), perceived age (5) and physical ability (6).

Shorter telomeres have been associated with lower lung function (7) and increased risk for lung diseases (8). However, this association is typically reported to be small and dependent of several health related habits and existence and progress of chronic diseases. COPD patients, asthmatics and smokers have shorter telomeres than healthy, non-smoking subjects (7-9). The associations found between LTL and lung function have led researchers to hypothesize that lung function may serve as a surrogate marker for biological aging, which is aggravated in lung diseases, possibly owing to intrinsic aging processes (7), oxidative stress and chronic inflammation (10). Although the development of an obstructive lung disease is strongly related to external factors such as tobacco smoking and air pollutants, lung function also deteriorates during healthy aging.

Both LTL (11) and lung function (12, 13) are known to be genetically regulated, and specific genes have been identified through genome-wide associations studies (14), but the known genes account for only a fraction of the heritable component estimated from family and twin data. Inter-individual variation in the amount and rate of decline with age both in lung function and in LTL has been less studied, although it is known to be affected by both genetic and environmental factors. Whether these markers of biological aging, i.e. LTL and lung function, share similar genetic influences is not known, but can be investigated in twin data with genetic modeling methods. Therefore the aim of this study was to examine whether, in community-living older female monozygotic and dizygotic twins, LTL and lung function share similar genetic and environmental factors. In addition, lung function variables were compared in twin pairs discordant for telomere length.

METHODS

Study design and participants

The study is part of The Finnish Twin Study on Aging (FITSA), which was established to investigate genetic and environmental effects on the disablement process in older women (15). The participants were recruited from the Finnish Twin Cohort, which comprises all same-sex twin pairs born before 1958 and with both co-twins alive in 1975 (16). Zygosity was determined at the baseline study in 1975 by a validated questionnaire (17) and confirmed in the FITSA study using DNA extracted from a venous blood sample by a battery of 10 highly polymorphic gene markers. In August 2000, there were 1,260 female twin pairs in the age group of 63–76 years who had

participated in the Finnish Twin Cohort in 1975. An invitation to participate in the FITSA study was sent on the basis of age and zygosity to a subsample of 414 twin pairs in this group (Supplement 1). Additional inclusion criteria were willingness to participate and the ability to travel to the laboratory of both co-twins in a pair. A total of 103 monozygotic (MZ) and 114 dizygotic (DZ) twin pairs (434 individuals) participated in the laboratory measurements. After excluding subjects with missing or incomplete measurements, total number of individuals included in the analysis was 386 (86 MZ and 92 DZ pairs).

The FITSA study was carried out according the Declaration of Helsinki on good clinical and scientific practice. The study protocol was approved by the Ethics Committee of the Central Hospital District of Central Finland (K-S shp: Dnro 24/2000). Before the laboratory examinations, the subjects were informed about the measurements and they gave their written informed consent.

Telomere length

LTL was determined from peripheral blood DNA by quantitative real-time polymerase chain reaction (qPCR), as described in Ahola et al. (18), with slight modifications (19). Briefly, as a single-copy reference gene, β-hemoglobin was used. Each plate included a DNA dilution series (0.5, 1.0, 2.0, 5.0, 10, 20, and 30 ng), which was used to create a standard curve and to perform absolute quantification of each sample. Samples and standard dilutions were analyzed in triplicate and samples with standard deviation >0.5 between triplicated were omitted from the analysis. Both the telomere and β-hemoglobin reactions were performed with the CFX 384 real-

time PCR detection system (Bio-Rad, Hercules, CA). We used the Bio-Rad CFX Manager v.1.1 software to perform rigorous quality control and to calculate the T/S (telomere to single-copy gene intensity) ratios for the samples in order to obtain the relative LTL. Each qPCR plate included five normal samples and one short LTL control sample that were used as calibrators to correct for possible batch effects between qPCR assay plates. The coefficient of variation for repeat measures was 6.37 % for the telomere reaction, 4.99 % for the β-hemoglobin reaction, and 6.97 % for the ratio (T/S). Of the subjects who participated in the laboratory measurements, 13 were excluded due to a missing or failed LTL measurement.

Lung function

Lung function measurements; forced expiratory volume in one second ($FEV_{1,}$ l/s), forced vital capacity (FVC, l), forced expiratory ratio (FEV_1/FVC) x 100 (%) and peak expiratory flow (PEF, l/s) were derived from spirometry. Percent-predicted (pp) values for FEV_1 and FVC were calculated according to the current European equations (Global Lungs Initiative, GLI) (20). Flow-volume spirometry including all values was performed according to international guidelines (American Thoracic Society, 1995) using an electronic device (Spiro 2000, Medikro Oy, Kuopio, Finland). Trained laboratory nurses made the measurements and guided the participants. Tests were performed in a standing position and nose clip was used. Subjects were encouraged to inhale maximally and to exhale into a mouthpiece connected to a flow transducer. Fast and slow expiratory maneuvers were practiced before actual measurements. Maneuvers were performed until at least two performances were acceptable and of these, the better performance was recorded as the subject's result. The spirometer

was calibrated daily and was accurate to within 1%. Reasons for not attending in spirometry (*n* = 25) were physician's recommendation owing to acute or chronic respiratory or other diseases, lack of time, and communication problems. In addition, 10 subjects provided incomplete spirometry results.

Additional measurements

Body mass index (BMI) was calculated as a function of measured weight and height (kg/m2). Subjects' pulmonary condition, smoking behavior and number of chronic diseases and medications were carefully evaluated with a questionnaire and confirmed by a physician during a clinical examination. Smoking was categorized into current smokers, previous smokers and never smokers (less than 5-10 packets in your lifetime). The current status of physical activity was assessed using the 7-point self-report scale by Grimby (21), with slight modifications. Participants were considered sedentary, if they reported no other activity than light walking once or twice a week. In all other cases, participants were considered physically active.

Statistical analysis

Descriptive statistics. Baseline statistics were analyzed by IBM SPSS statistics 22.0 software. Data are presented as means with standard deviations (SD). P-values for differences between individuals from the MZ versus DZ twin pairs were derived from design-corrected Student's t-test for independent samples or chi-square test. Associations between LTL and lung function were tested with bivariate correlations and within-pair dependency of twin individuals was taken into account with cluster option in Stata 14 (Williams, Biometrics 2000)..

The genetic modeling is based on the fact that MZ twins share all their genes while DZ twins share on average 50% of their segregating genes. Greater similarity observed between MZ twin pairs compared to DZ twin pairs is evidence for genetic influence on the trait (22). The observed variance in phenotype can be decomposed into four sources of variance: additive genetic (A), nonadditive genetic dominance (D), shared environment (C) and unique (nonshared) environment (E) (22). The expected correlation for A, D and C between the twins in MZ pairs is 1.0, while for DZ pairs the expected correlations are: for A 0.5, for D 0.25 and for C 1.0. By definition, the effects of E are uncorrelated in both MZ and DZ twins, and because E includes stochastic effects and measurement error its value can never be zero (23). For environmental effects, shared effects (C) represent similar environmental sources of variance, while (E) measures the environmental variance and error unique to each trait. C and D cannot be modeled simultaneously, as these components are confounders in designs that only include twins reared together.

Prior genetic analysis variable distributions were evaluated and transformed when necessary. LTL was log-transformed and standardized to rescale the variable metric suitable for analysis. None of the lung function variables deviated from the Gaussian distribution. Intraclass correlation coefficients (ICC) and 95% confidence intervals (95% CI) were computed separately for the MZ and DZ twin pairs to estimate the level of within-pair similarity and the ratios of the MZ and DZ correlations. In the absence of dominance or common environmental effects heritability is approximately twice the difference between the MZ and DZ correlations. Genetic

dominance effects can be assumed to be present if the initial correlation analysis shows that the MZ correlations are more than twice as high as the DZ correlations. In these cases, the full ADE model was fitted to the data. If the ratio was less than two, the dominance effect was assumed to be absent and the ACE model was applied. The significance of the contribution of the individual variance components to total trait variance were tested in either the full ADE or ACE model, using submodels in which one or more of the variance components were fixed to zero. The likelihood ratio test and information criteria were used to identify the most parsimonious and best-fitting model to explain the observed pattern of twin similarity in the MZ and DZ twin pairs and to compare the difference in fit between models. Genetic dominance effects in the absence of additive effects (DE-model) were considered unsupported by the twin design and were thus not fitted. To quantify possible genetic and shared and unique environmental associations between LTL and lung function, the variables were first modeled separately, using univariate models. Next, to evaluate whether the LTL and lung function estimates share genetic or environmental components or whether the genetic or environmental effects are specific to each measurement, the bivariate correlated factors model was built (22, 24). Genetic and environmental correlations were estimated to assess the sharing of genetic and environmental sources between the LTL and lung function variables. The model parameters were estimated and bootstrap confidence intervals calculated in Mplus software, version 7 (25).

As a known confounder for LTL and lung function, age was taken into account as a covariate in all the univariate and bivariate models. For LTL, current and former

smoking and for lung function height and current and former smoking were considered potential confounders. The regression coefficient for height was significant in the FEV_1, FVC and PEF models, but had minor effects on the variance components (data not shown) and was therefore excluded from the analysis. Regression coefficients for current and former smoking were modeled separately in each of the univariate and bivariate models. Missing data in lung function was mainly due to subjects' inability to perform the spirometry measurement because of acute or chronic lung function problems or other diseases. To test if missing data in lung function had significant effects on the results, a sensitivity analysis was performed by modeling missing data not at random into the bivariate model. This had negligible effects on the results (data not shown). Statistical significance was set at level $p<0.05$ in all analyses.

RESULTS

Characteristics: The MZ and DZ twins did not differ with respect to the means and variances of age, anthropometric variables or lung function (Table 1). Prevalence of asthma, low lung function status (FEV_1/FVC ratio below 70%), current and former smoking, number of chronic diseases, level of physical activity and use of prescriptive medicines were similar in the MZ and DZ twins. In all, higher chronological age was associated with shorter LTL (r=-0.128, p=0.034).

Pairwise similarity i.e. intraclass correlation coefficients (ICC). For LTL, the pairwise ICCs were greater in the MZ twins (r=0.534, 95% CI 0.365-0.669) compared to DZ twins (r=0.372, 95% CI 0.182-0.535), indicating a genetic influence on LTL. The ICCs

were notably higher in the MZ (FVC; r=0.666 95% CI 0.530-0.769, FEV1/FVC; 0.367 95% CI 0.170-0.536 and PEF; 0.657 95% CI 0.518-0.762) than DZ twin pairs (0.318 95% CI 0.123 -0.490-0.027 95% CI -0.177-0.229 and 0.142 95% CI -0.063-0.336, respectively), indicating genetic influences on the variance of the lung function measures. ICC in FEV_1 was 0.687 (95% CI 0.558-0.784) in the MZ pairs and 0.207 (95% CI 0.004-0.394) in the DZ twin pairs, indicating the possibility that FEV_1 has a combined genetic-environment make-up that is difficult to disentangle in the twin study design.

Bivariate correlations. In the whole sample, longer LTL was associated with higher FEV_1 (r=0.104, p=0.041), but no statistically significant correlations were found between LTL and the other measured or percent-predicted lung function variables. The observed correlation between LTL and FEV_1 raises the possibility that these phenotypes are affected by shared genetic or environmental factors and thus these variables were investigated by univariate and bivariate genetic models.

Genetic modeling – Univariate models. The analysis revealed that the model including additive genetic effects and unique environment (AE) showed the best fit to both the LTL and lung function data. For LTL, additive genetic factors explained 62 % (95% CI 50 to 72) and unique environmental factors 38 % (95% CI 28 to 50) of the variance (Table 2). The ACE and CE models yielded a model fit very similar to that of the AE model in LTL. For FEV_1, FVC and PEF, the corresponding estimates for A varied between 65 and 67%, and for E between 33 and 35% (Table 3). For FEV_1/FVC, the

best fitting model consisted of 30 % (95% CI 7 to 48) additive genetic effects and 70 % (95% CI 52 to 93) unique environmental effects.

Genetic modeling – Bivariate models. The genetic correlation between LTL and FEV_1 was 0.18 (95% CI -0.19 to 0.64) and the environmental correlation was -0.10 (95% CI -0.84 to 0.55) (Figure 1). Current and former smoking had minor effects on the model (Table 4).

DISCUSSION

Classical twin design used in this study allowed us to evaluate the approximate magnitude of the genetic and environmental factors behind suggested biological aging markers: LTL (1, 26) and lung function (27). We observed that both LTL and lung function are genetically influenced. The majority of the variance of LTL and lung function was determined by additive genetic factors, but unique environmental factors also played a significant role. This study lacks power to resolve if the observed weak association between LTL and FEV_1 can be explained by either shared genetic or environmental factors. However, results revealed that it is more likely that shared genes explain the associations between LTL and lung function. These preliminary findings need to be confirmed with larger twin samples or genetic association studies.

A rough estimate of heritability can be drawn on the basis of the ratio of intraclass correlation coefficients between the MZ and DZ twin pairs. The components of the

variance can be more precisely estimated in univariate genetic models. Several studies have investigated the heritability of LTL in twin and family studies. As in our study, the majority of these studies have indicated that LTL has a strong genetic component in (11, 28, 29). A recent meta-analysis reported a heritability estimate of 70% for LTL (11). Other studies with smaller samples have yielded in heritability estimates ranging from 34% to 82% (11). It has been suggested that the variation in estimates is due to different samples and sample sizes (11). The age-adjusted LTL heritability estimates observed in this study (62%) were slightly smaller than the rates of 84% and 82% reported by Jeanclos (29) and Vasa-Nicotera (28), respectively. Heritability estimates are population-specific estimates, i.e., they are dependent on the genetic make-up of the population, and also on the presence or absence of environmental factors affecting telomere length in the study sample.

LTL is a complex trait that is affected by many factors. In addition to a major genetic component, several epigenetic, lifestyle and environmental factors may also play a role. In general, in many biological variables, heritability and unique environmental effects increase, while shared environmental factors decrease, with advancing age (30). Recent studies have shown that heritability and the early life environment are the main determinants of LTL throughout the human life course, i.e., having short or long telomeres, is determined before adulthood (31). However, it has been suggested that the small proportion of telomere attrition that occurs in adulthood is explained by unique environmental factors (31). Lifestyle, mental stress, socioeconomic status and nutrition and energy intake may play a role in LTL attrition during adulthood. Our sensitivity analysis suggested that physical activity and education did not affect the

current results (data not shown), however several lifestyle factors, mental stress and nutrition could have an effect that we were not able to control within this design.

Our findings on the heritability of lung function are also in line with those previously reported (12, 32, 33). In the current sample, additive genetic factors accounted for 65% of the variability in FEV_1 and 67% of FVC. Very similar heritability estimates of 61% and 67% for FEV_1 in older twins have been reported earlier (12, 34). In addition, our estimate of the genetic contribution to FVC is comparable with the estimate (68%) reported by Tarnoki et al. (32) and slightly higher than that (55%) reported by Ingebrigtsen and co-workers (12). Although, the interaction of genes and smoking may influence lung function (35), our data showed that current or former smoking had minor effects on the heritability estimates of lung function. In an earlier analysis of the present sample, conducted with non-smokers only, the heritability estimates were lower for most of the lung function parameters (36).

A significant, although weak, positive correlation was found between LTL and FEV_1, which indicates that longer LTLs are associated with better lung function. However, this association with LTL was not observed with the other lung function variables. It is known that, with aging, FEV_1 begins to decline earlier and at a much faster rate than, for example, FVC, which might explain our findings (37). In a large meta-analysis the associations between LTL and lung function variables have also been reported to be stronger in asthmatics and chronic obstructive lung disease patients than healthy controls (7). Our subjects were healthy older females who were able to participate in the laboratory measurements and very few of them were smokers. This might

explain the relatively small associations observed between LTL and the lung function variables. In this study, we performed bivariate genetic modeling for the first time. The analysis, in light of the modest correlations and rather small sample size, was not able to resolve if the present phenotypic correlation between LTL and FEV_1 was explained by shared genetic or environmental factors. However, the genetic correlation point estimate was positive and greater than that of the environmental components, suggesting that in a larger sample, the phenotypic correlation might be accounted for by genetic factors in common.

The association observed between LTL and lung function in the present and earlier papers (7, 8) suggests the presence of common factors involving both the telomere biology and lung function. Of the potential unique environmental factors, tobacco smoke is known to promote aging processes and cellular senescence (38) and telomere shortening (8) in addition to its known damaging effects on lung function (39). We found that current smoking, but not previous smoking was significantly associated with lung function variables FEV_1, PEF and the FEV_1/FVC ratio, but not FVC or LTL, and that smoking had a minor influence on the genetic and environmental components of the lung function variables, as observed previously (12). Our data included only a few smokers and there was a large heterogeneity in smoking exposure and its amount and duration. The Interactions between smoking and genetic and/or environmental factors are very complex and difficult to model, and our sample size did not permit such modeling. Another possible shared factor is chronic inflammation, which is known to increase with aging. We have previously reported stronger associations between LTL and lung function among asthmatics and

COPD patients than lung-healthy subjects (7). It is possible that LTL and lung function are somehow related through intrinsic age-related processes which are aggravated by, for example, chronic inflammation either locally in the lung tissue or systemically in the whole body.

To our knowledge this is the first study that has attempted to model the genetic and environmental associations between LTL and lung function in a twin design. A comprehensive sample of subjects from the Finnish twin cohort study was recruited and all the measurements were carefully performed according to internationally standardized practices at the same university laboratory. Some criticism has been leveled at the qPCR method used in this study, although it is widely used and has good repeatability (40). The study sample is representative of Finnish older women aged 65 to 70 year old, the majority of whom were non-smokers, and therefore the results cannot be generalized to other populations. Moreover, the analysis was performed within a cross-sectional design and conclusions on the causality of LTL attrition and decreased lung function cannot be drawn.

Our data suggest for the first time, that in spite of the associations observed in other studies and our team (7, 8), LTL and lung function do not appear to share very strong genetic or environmental factors in common. Future research is needed to confirm our findings and to evaluate what factors might affect both telomere attrition and estimates of lung function. Possible confounders could be related to diseases and other individual stress and health-related factors and processes that are regulated by complex gene-environment associations.

Funding

The Finnish Twin Study of Aging (FITSA) was funded by the Ministry of Education, Academy of Finland, and the EC FP5 GenomEUtwin project. E.S. is supported by a post doc research grant from the Academy of Finland (grant number 260001). J.K. has been supported by the Academy of Finland (grants 265240, 263278).

Acknowledgments

The authors acknowledge Dr. Iiris Hovatta and Ms. Jenni Lahtinen for carrying out telomere length analysis by qPCR. The Gerontology Research Center is a joint effort between the University of Jyväskylä and the University of Tampere.

Figure legend

Figure 1. Correlated factors model for leukocyte telomere length (LTL) and forced expiratory volume in one second (FEV_1). A, additive genetic effect; E, unique environment; r^A, genetic correlation, r^E, environmental correlation.

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Table 1. Characteristics of the study participants by zygosity status.

Variable*	MZ (n=186)	DZ (n=200)	P-value
Age (yr)	68.1 (3.7)	68.6 (3.1)	0.16
Height (m)	158.0 (6.5)	159.0 (5.8)	0.21
Body mass (kg)	69.3 (11.7)	70.4 (12.3)	0.44
BMI (kg/m^2)	27.9 (4.8)	27.9 (4.7)	0.99
Telomere length	0.88 (0.18)	0.91 (0.20)	0.34
Lung function			
FEV_1 (L/s)	2.3 (0.5)	2.2 (0.5)	0.12
FVC (L)	2.9 (0.6)	2.8 (0.6)	0.19
FEV_1/FVC (%)	72.9 (8.9)	74.8 (9.6)	0.058
PEF (L/s)	5.5 (1.4)	5.5 (1.4)	0.90
Standardized lung function			
FEV_1-pp (%)	104.9 (19.6)	100.5 (22.7)	0.085
FVC-pp (%)	104.5 (17.8)	100.7 (21.6)	0.14
Pulmonary condition			
Astma diagnosis (n, %)	12 (6.5)	17 (8.5)	0.51
FEV_1/FVC below 70% (n, %)	24 (12.9)	26 (13.0)	0.98
Current smoking (n, %)	8 (4.3)	13 (6.5)	0.42
Former smoking (n, %)	20 (10.8)	11 (5.5)	0.060
Health			
Number of chronic diseases	2.0 (1.5)	2.2 (1.4)	0.92
Number of medications	1.8 (2.0)	2.2 (1.9)	0.065
Physically active (n,%)	137 (73.7)	144 (72.0)	0.75

*Variables are presented as mean and standard deviation unless otherwise indicated. MZ, monozygotic; DZ, dizygotic; BMI, body mass index; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; PEF, peak expiratory flow; pp, percent predicted.

Table 2. Univariate models for telomere length.

	Model Fit			**Parameter Estimates and Their 95 % Confidence Intervals**						
	χ^2	**df**	**BIC**	a^2	c^2	d^2	e^2	**age**	s_1	s_2
ACE	2.35	9	1057	37 (0, 68)	23 (0, 57)	-	**40** (29, 52)	**-0.04** (-0.08, -0.01)	-	-
AE	3.85	10	1053	**62** (50, 72)	-	-	**38** (28, 50)	**-0.04** (-0.08, -0.01)	-	-
CE	5.68	10	1055	-	**50** (37, 61)	-	**50** (39, 63)	**-0.04** (-0.08, -0.01)	-	-
E	52.8	11	1097	-	-	-	**100**	**-0.04** (-0.08, -0.01)	-	-
ACE	28.6	23	959	40 (0, 67)	18 (0, 54)	-	**42** (30, 54)	**-0.04** (-0.08,-0.01)	0.03 (-0.33, 0.42)	0.10 (-0.21, 0.37)
AE	29.5	24	954	**60** (48, 71)	-	-	**40** (30, 52)	**-0.04** (-0.08, -0.01)	0.03 (-0.34, 0.43)	0.12 (-0.17, 0.39)
CE	32.3	24	957	-	**47** (34, 58)	-	**53** (42, 66)	**-0.04** (-0.08, -0.01)	0.04 (-0.31, 0.40)	0.06 (-0.26, 0.35)
E	76.7	25	996	-	-	-	**100**	**-0.04** (-0.08, -0.01)	0.00 (-0.41, 0.43)	0.18 (-0.18, 0.53)

Dark grey: Smallest BIC (best fitting model); Light grey: Probability of minimizing information loss relative to best fitting model of more than 0.05. a^2, c^2, e^2 are percent estimates of variance components. Regression coefficients for age (age), current smoking indicator (s_1) and former smoking indicator (s_2) are presented in the table. Telomere length was modified by multiplying the logarithm of the original variable by five. Bootstrap 95 % confidence intervals were based on 10 000 draws. Statistical significance at the 0.05 level is shown in bold typeface.

Table 3. Univariate models for lung function.

	Model Fit			Parameter Estimates and Their 95 % Confidence Intervals						
	χ^2	df	BIC	a^2	c^2	d^2	e^2	age	s_1	s_2
FEV_1										
ADE	7.88	9	499	3 (0, 59)	-	64 (0, 79)	**33** (20, 51)	**-0.03** (-0.05, -0.02)	-	-
AE	10.5	10	496	**65** (46, 79)	-	-	**35** (21, 54)	**-0.03** (-0.05, -0.02)	-	-
E	59.0	11	540	-	-	-	**100**	**-0.04** (-0.05, -0.02)	-	-
ADE	29.0	23	443	0 (0, 46)	-	**67** (12, 79)	**33** (20, 52)	**-0.04** (-0.06, -0.02)	**-0.30** (-0.52, -0.07)	0.04 (-0.15, 0.21)
AE	32.9	24	442	**64** (44, 79)	-	-	**36** (21, 56)	**-0.04** (-0.06, -0.02)	**-0.29** (-0.51, -0.05)	0.03 (-0.16, 0.20)
E	78.3	25	482	-	-	-	**100**	**-0.04** (-0.06, -0.02)	**-0.35** (-0.56, -0.09)	-0.05 (-0.32, 0.17)
FVC										
ADE	7.67	9	649	50 (0, 73)	-	17 (0, 71)	**33** (22, 45)	**-0.04** (-0.06, -0.01)	-	-
AE	7.87	10	644	**67** (54, 77)	-	-	**33** (23, 46)	**-0.04** (-0.06, -0.01)	-	-
E	60.8	11	691	-	-	-	**100**	**-0.04** (-0.06, -0.01)	-	-
ADE	30.2	23	579	39 (0, 70)	27 (0, 73)	-	**34** (23, 47)	**-0.04** (-0.06, -0.02)	**-0.24** (-0.46, -0.02)	0.04 (-0.19, 0.26)
AE	30.7	24	575	**65** (52, 77)	-	-	**35** (23, 48)	**-0.04** (-0.06, -0.02)	**-0.24** (-0.45, -0.01)	0.04 (-0.19, 0.25)
E	81.3	25	620	-	-	-	**100**	**-0.04** (-0.07, -0.02)	**-0.29** (-0.55, -0.03)	-0.08 (-0.37, 0.18)
FEV_1/FVC										
ADE	18.8	9	2826	0 (0, 31)	-	36 (0, 54)	64 (46, 87)	**-0.37** (-0.67, -0.07)	-	-
AE	21.2	10	2823	**30** (7, 48)	-	-	**70** (52, 93)	**-0.37** (-0.66, -0.06)	-	-
E	30.7	11	2827	-	-	-	**100**	**-0.35** (-0.66, -0.04)	-	-
ADE	44.4	23	2608	0 (0, 32)	-	**37** (0, 56)	**63** (44, 89)	**-0.39** (-0.74, -0.07)	**-7.17** (-12.58,-1.69)	-1.04 (-5.10, 2.77)
AE	46.9	24	2605	**30** (5, 49)	-	-	**70** (51, 95)	**-0.39** (-0.73, -0.06)	**-7.12** (-12.48,-1.71)	-1.16 (-5.28, 2.75)
E	55.7	25	2609	-	-	-	**100**	**-0.38** (-0.73, -0.04)	**-7.20** (-12.43,-1.91)	-1.05 (-5.59, 3.09)
PEF										
ADE	8.22	9	1323	0 (0, 63)		**68** (0, 79)	**32** (21, 48)	**-0.07** (-0.12, -0.02)	-	-
AE	12.2	10	1321	**65** (47, 79)	-	-	**35** (22, 53)	**-0.07** (-0.12, -0.02)	-	-
E	53.8	11	1358	-	-	-	**100**	**-0.07** (-0.12, -0.02)	-	-
ADE	22.8	23	1186	0 (0, 44)	-	**64** (16, 77)	**35** (22, 53)	**-0.07** (-0.12, -0.02)	**-0.84** (-1.28, -0.39)	0.18 (-0.40, 0.80)
AE	28.5	24	1187	**60** (39, 76)	-	-	**40** (24, 61)	**-0.07** (-0.12, -0.02)	**-0.78** (-1.24, -0.33)	0.17 (-0.39, 0.78)
E	65.7	25	1219	-	-	-	**100**	**-0.08** (-0.12, -0.03)	**-0.97** (-1.48, -0.30)	0.15 (-0.62, 0.80)

FEV_1, Forced expiratory volume in 1 s; FVC, Forced vital capacity; PEF, peak expiratory flow. Dark grey: Smallest BIC (best fitting model); Light grey: Probability of minimizing information loss relative to best fitting model of more than 0.05. a^2, d^2, e^2 are percent estimates of variance components. Regression coefficients for age (age), current smoking indicator (s_1) and former smoking indicator (s_2) are presented in the table. Statistical significance at the 0.05 level is shown in bold typeface.

Table 4. Bivariate correlated factors model for telomere length and FEV_1.

Model Fit					Parameter Estimates and Their 95 % Confidence Intervals						
Model	**χ^2**	**df**	**BIC**	**Outcome**	**a^2**	**e^2**	**age**	**s_1**	**s_2**	**r_a**	**r_e**
1	20	26	1558	Telomere length	**62** (51, 72)	**38** (28, 50)	**-0.04** (-0.08, -0.01)	--	--	0.18 (-0.19, 0.64)	-0.10 (-0.84, 0.55)
				FEV_1	**66** (47, 79)	**34** (21, 54)	**-0.03** (-0.05, -0.02)	--	--		
2	126	74	1663	Telomere length	**62** (50, 72)	**38** (28, 50)	**-0.04** (-0.08, -0.01)	-0.07 (-0.46, 0.33)	0.07 (-0.22,0.34)	0.19 (-0.18, 0.64)	-0.13 (-0.86, 0.51)
				FEV_1	**66** (45, 80)	**34** (20, 55)	**-0.03** (-0.05, 0.02)	**-0.29** (-0.50, -0.08)	0.03 (-0.05, 0.02)		

Note. FEV_1, Forced expiratory volume in 1 s. a^2 and e^2 are percent estimates of variance components. Regression coefficients for age (age), current smoking indicator (s_1) and former smoking indicator (s_2) are presented in the table. Telomere length was modified by multiplying the logarithm of the original variable by five. Bootstrap 95 % confidence intervals were based on 10 000 draws. Statistical significance at the 0.05 level is shown in bold typeface.