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Phenotypic and genetically predicted leucocyte telomere length and lung cancer risk in the prospective UK Biobank

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ABSTRACT

We investigated phenotypic leucocyte telomere length (LTL), genetically predicted LTL (gTL), and lung cancer risk among 371 890 participants, including 2829 incident cases, from the UK Biobank. Using multivariable Cox regression, we found dose-response relationships between longer phenotypic LTL (p-trend$_{continuous}$=2.6×10^{-5}), longer gTL predicted using a polygenic score with 130 genetic instruments (p-trend$_{continuous}$=4.2×10^{-10}), and overall lung cancer risk, particularly for adenocarcinoma. The associations were prominent among never smokers. Mendelian Randomization analyses supported causal associations between longer telomere length and lung cancer (HR$_{per\ 1\ SD\ gTL}$=1.87, 95% CI: 1.49 to 2.36, p=4.0×10^{-7}), particularly adenocarcinoma (HR$_{per\ 1\ SD\ gTL}$=2.45, 95%CI: 1.69 to 3.57, p=6.5×10^{-6}).

INTRODUCTION

Telomere length reflects the cumulative burden of exposures, endogenous factors, and age. We investigated phenotypic leucocyte telomere length (LTL), telomere length predicted using a polygenic score (PGS; gTL), and lung cancer risk in the United Kingdom (UK) Biobank. Further, we examined the associations by histology and subgroups defined by smoking status and sex. To evaluate causal relationships, we conducted Mendelian Randomization (MR) analyses using 130 genetic instruments that predict longer LTL.[1 2] Our study could help identify high-risk subpopulations that do not have but may benefit from lung cancer screening.

MATERIALS AND METHODS

Study population

The UK Biobank is described previously[3] and in the *Supplementary Materials*. Among 502 409 participants at baseline, we excluded 372 subjects with discrepancies between self-reported and genetic sex; 46 577 with any cancer diagnosis; 57 253 with respiratory diseases; and 4779 with hematologic/immunologic disorders. Our analytic dataset had 393 428 participants.

Follow-up time started at the visit date to the assessment centres in 2006–2010 and ended at the date of primary incident lung cancer diagnosis, death, or administrative censoring. Lung cancer diagnosis was defined by International Classification of Diseases 10th revision (ICD-10) codes C34.0-C34.9. ICD-O-3 code 8140 defined adenocarcinoma (LUAD), while 8052, 8084, 8073, and 8083 defined squamous cell carcinoma (SCC).

LTL measurements

The DNA extraction, multiplex quantitative polymerase chain reaction (qPCR) assay, and quality control procedures were previously described.[4] The ratio of telomere (T) to single-copy gene (S) copy number (*'T/S ratio'*) reflects the average telomere abundance across all chromosomes in leucocytes of an individual, which was further adjusted for batch variation (*'adjusted relative* T/S ratio').[4]

PGS for longer gTL and MR analyses

Using a one-sample approach,[1] we constructed a weighted PGS that predicted longer gTL with 130 single nucleotide polymorphisms (SNPs) identified among Europeans in the UK Biobank. These SNPs explained 4.54% of the variance in LTL.[1] We note that LTL is affected by the exposome, which can influence LTL variation but unlikely bias the identification of LTL-related SNPs. The SNPs were linkage disequilibrium pruned ($r^2 > 0.01$), reached genome-wide significance ($p<8.31\times10^{-9}$), and included in previous MR analyses.[1] The weights were based on reported Z-standardised beta-estimates.[1] To estimate causal associations between gTL and lung cancer and its subtypes among Europeans, we conducted MR analyses using the same 130 SNPs with MR-PRESSO in R. The HR from MR analyses reflect increased lung cancer risk per one SD increase in gTL.[1]

Statistical analyses

Cox regression was used to estimate HRs and 95% CI of incident lung cancer in relation to quartiles (Q) of phenotypic LTL ('*adjusted relative* T/S Ratio'; Q1:<0.74; Q2:0.74 to<0.82; Q3:0.82 to<0.91; Q4:≥0.91), adjusted for age, sex, race/ethnicity, detailed smoking history/intensity, assessment centre, body mass index, Townsend Deprivation Index, alcohol intake, secondhand smoke exposure, and leucocyte differentials. Follow-up time was the timescale. LUAD and SCC were analysed separately. We fitted separate Cox models with gTL as the main effect among Europeans, without leucocyte differential adjustment.

Linear trends were estimated using continuous gTL and log-transformed phenotypic LTL.

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Table 1 Associations between measured phenotypic leucocyte telomere length and lung cancer risk in 371 890 participants from the UK Biobank

Quartile	Adjusted relative T/S Ratio Cutoffs	No. of incident cases	HR	95% CI lower	95% CI upper	p-value	
		Overall lung cancer (2829 cases)					
1	<0.74	812	1.00				
2	0.74 to<0.82	666	1.00	0.90	1.11	0.97	
3	0.82 to<0.91	626	1.08	0.97	1.19	0.18	
4	≥0.91	612	1.27	1.15	1.42	8.9×10^{-6}	*
					p-trend	2.6×10^{-5}	*
		II) Lung adenocarcinoma (1078 cases)					
		No. of incident cases	**HR**	**95% CI Lower**	**95% CI Upper**	**p-value**	
1	<0.74	254	1.00				
2	0.74 to<0.82	256	1.19	1.00	1.41	0.06	
3	0.82 to<0.91	237	1.23	1.03	1.47	0.02	*
4	≥0.91	292	1.78	1.50	2.12	4.0×10^{-11}	*
					p-trend	6.6×10^{-10}	*
		III) Lung squamous cell carcinoma (487 cases)					
		No. of incident cases	**HR**	**95% CI Lower**	**95% CI Upper**	**p-value**	
1	<0.74	164	1.00				
2	0.74 to<0.82	118	0.95	0.75	1.21	0.68	
3	0.82 to<0.91	111	1.06	0.83	1.35	0.66	
4	≥0.91	72	0.88	0.67	1.17	0.38	
					p-trend	0.48	

Multivariable Cox regression models were used to estimate HR and 95% CI of incident lung cancer in relation to quartiles of *adjusted relative* T/S Ratio (ie, measured phenotypic LTL), adjusted for age at recruitment (continuous), sex (men vs women), race/ethnicity/ancestry (reference: European), detailed smoking history/intensity (28 categories, reference: never smokers), study assessment centre, BMI (reference: ≥18.5 to <25.0 kg/m^2), Townsend Deprivation Index (continuous), alcohol intake (reference: never drinker), exposure to secondhand smoke (ever vs never), and leucocyte subtype differentials (% lymphocytes, neutrophils, eosinophils, basophils and monocytes). P-trends were estimated using continuous log-transformed *adjusted relative* T/S ratio.

*P-values<0.05 were considered statistically significant. A total of 371 890 participants with complete data on independent variables were included in the analyses. Discrepancy in counts was due to missing LTL data.

Stratified analyses were conducted among subgroups defined by smoking status (never, former, current) and sex. We tested for multiplicative effect modification of LTL-lung cancer associations by smoking status and sex using cross-product terms. Heterogeneity between HR estimates was assessed using Z-score sign tests.

RESULTS

Phenotypic LTL and lung cancer

There were 2829 incident lung cancer cases, including 1078 LUAD and 487 SCC, diagnosed over the 12.36±1.64 SD year follow-up. The average time between LTL measurements and lung cancer diagnosis was 6.54±3.22 SD years. We found a strong dose-response relationship between longer phenotypic LTL and increased lung cancer risk (p-trend$_{continuous}=2.6\times10^{-5}$), particularly LUAD (p-trend$_{continuous}=6.6\times10^{-10}$) but not SCC (table 1; p-difference$_{LUAD\ vs.\ SCC}=6.7\times10^{-5}$). Findings were similar among Europeans and when including participants with chronic respiratory diseases at enrollment (online supplemental table 1).

We observed multiplicative effect modification of the LTL-lung cancer association by smoking, with attenuation among former (p-interaction=0.008) and current smokers (p-interaction=0.008) vs never smokers. We did not detect an interaction between phenotypic LTL and sex (p-interaction=0.909). Among subgroups, we found associations between longer phenotypic LTL and increased lung cancer risk among never smokers (p-trend=3.3×10^{-7}), including never-smoking women (p-trend=4.0×10^{-5}) and men (p-trend=2.0×10^{-3}), as well as former-smoking women (p-trend=5.7×10^{-4}) (table 2). When analysing LUAD, the trends were consistent with overall lung cancer (online supplemental table 2). Due to sparse data, we could not analyse SCC among never smokers and consistent associations were not detected in other subgroups (online supplemental table 2)

PGS and MR analyses among Europeans

In PGS analyses, we found strong dose-response relationships between longer gTL and lung cancer risk (p-trend=4.2×10^{-10}), particularly LUAD (p-trend=2.1×10^{-8}) but not SCC (figure 1). We did not detect multiplicative interactions between gTL and smoking or sex (p-interactions>0.05).

In MR analyses, we found evidence for causal associations between longer gTL and lung cancer ($HR_{per\ 1\ SD\ gTL}=1.87$, 95% CI: 1.49 to 2.36, p=4.0×10^{-7}), particularly LUAD ($HR_{per\ 1\ SD\ gTL}=2.45$, 95% CI: 1.69 to 3.57, p=6.5×10^{-6}) but not SCC ($HR_{per\ 1\ SD\ gTL}=1.19$, 95% CI: 0.74 to 1.93, p=0.47; p-difference$_{LUAD\ vs.\ SCC}=0.02$). No horizontal pleiotropy was detected for any SNP.

DISCUSSION

We confirm and expand on previous studies with important findings among subpopulations defined by smoking and sex.

Table 2 Associations between measured phenotypic leucocyte telomere length and overall lung cancer risk in the UK Biobank among subgroups defined by sex and smoking status

Quartile	Adjusted relative T/S Ratio Cutoffs	No. of incident cases	HR	95% CI lower	95% CI upper	p-value	
I) NEVER SMOKERS		424 cases / 205 237 subjects					
1	<0.74	91	1.00				
2	0.74 to<0.82	74	0.85	0.63	1.16	0.31	
3	0.82 to<0.91	109	1.33	1.00	1.75	4.9×10^{-2}	*
4	≥0.91	130	1.72	1.31	2.26	9.9×10^{-5}	*
					p-trend	3.3×10^{-7}	*
II) FORMER SMOKERS		1254 cases / 125 995 subjects					
1	<0.74	352	1.00				
2	0.74 to<0.82	295	1.01	0.87	1.18	0.88	
3	0.82 to<0.91	283	1.13	0.96	1.32	0.14	
4	≥0.91	272	1.33	1.13	1.56	5.3×10^{-4}	*
					p-trend	3.0×10^{-3}	*
III) CURRENT SMOKERS							
		1120 cases / 38 808 subjects					
1	<0.74	360	1.00				
2	0.74 to<0.82	288	1.05	0.90	1.23	0.53	
3	0.82 to<0.91	227	0.96	0.81	1.13	0.61	
4	≥0.91	206	1.09	0.92	1.30	0.33	
					p-trend	0.49	
IV) NEVER SMOKING WOMEN		271 cases / 118 044 subjects					
Quartile	Adjusted Relative T/S Ratio Cutoffs	No. of incident cases	HR	95% CI Lower	95% CI Upper	p-value	
1	<0.74	58	1.00				
2	0.74 to<0.82	37	0.62	0.41	0.94	0.02	*
3	0.82 to<0.91	70	1.18	0.83	1.67	0.36	
4	≥0.91	91	1.55	1.11	2.17	0.01	*
					p-trend	4.0×10^{-5}	*
V) NEVER SMOKING MEN		153 cases / 87 193 subjects					
1	<0.74	33	1.00				
2	0.74 to<0.82	37	1.33	0.83	2.12	0.24	
3	0.82 to<0.91	39	1.60	1.00	2.55	0.05	
4	≥0.91	39	2.03	1.27	3.26	3.3×10^{-3}	*
					p-trend	2.0×10^{-3}	*
VI) FORMER SMOKING WOMEN		545 cases / 59 763 subjects					
1	<0.74	114	1.00				
2	0.74 to<0.82	120	1.09	0.85	1.41	0.50	
3	0.82 to<0.91	135	1.30	1.01	1.67	0.04	*
4	≥0.91	147	1.57	1.23	2.02	3.3×10^{-4}	*
					p-trend	5.7×10^{-4}	*
VII) FORMER SMOKING MEN		709 cases / 66 232 subjects					
1	<0.74	238	1.00				

Continued

Table 2 Continued

Quartile	Adjusted Relative T/S Ratio Cutoffs	No. of incident cases	HR	95% CI Lower	95% CI Upper	p-value	
2	0.74 to<0.82	175	0.99	0.81	1.20	0.89	
3	0.82 to<0.91	148	1.03	0.84	1.27	0.75	
4	≥0.91	125	1.19	0.96	1.48	0.12	
					p-trend	0.32	
VIII) CURRENT SMOKING WOMEN		455 cases / 16 649 subjects					
1	<0.74	145	1.00				
2	0.74 to<0.82	106	0.81	0.63	1.04	0.09	
3	0.82 to<0.91	93	0.73	0.56	0.95	0.02	*
4	≥0.91	99	0.92	0.71	1.20	0.55	
					p-trend	0.41	
IX) CURRENT SMOKING MEN		665 cases / 22 159 subjects					
1	<0.74	215	1.00				
2	0.74 to<0.82	182	1.23	1.01	1.50	0.04	*
3	0.82 to<0.91	134	1.13	0.91	1.41	0.26	
4	≥0.91	107	1.19	0.94	1.51	0.15	
					p-trend	0.14	

Multivariable Cox regression models were used to estimate HR and 95% CI of incident overall lung cancer in relation to quartiles of *adjusted relative* T/S Ratio (ie, measured phenotypic LTL), adjusted for age at recruitment (continuous), race/ethnicity/ancestry (reference: European), detailed smoking history/intensity when analysing former and current smokers, study assessment centre, BMI (reference: ≥18.5 to <25.0 kg/m^2), Townsend Deprivation Index (continuous), alcohol intake (reference: never drinker), exposure to secondhand smoke (ever vs never), and leucocyte subtype differentials (% lymphocytes, neutrophils, eosinophils, basophils and monocytes). P-trends were estimated using continuous log-transformed *adjusted relative* T/S ratio.
*P-values<0.05 were considered statistically significant. Discrepancy in counts were due to missing LTL data.

Previously, we reported associations between longer phenotypic LTL and increased lung cancer risk when pooling three nested case-control studies, particularly for LUAD among Europeans.[5] Those findings were corroborated in separate studies of Chinese[6] and heavy-smoking European populations.[7] Our PGS and MR analyses were in agreement with studies that found associations between longer gTL and increased lung cancer risk.[8–10] In particular, our MR analyses support causal associations between gTL and lung cancer. The magnitude of our gTL and phenotypic LTL effects were similar; suggesting that the genetic component of LTL captured by gTL is an effective surrogate marker, despite explaining a small proportion of variance. Our findings support longer pre-diagnostic LTL in processes relevant to lung carcinogenesis, prominently among non-smokers. Longer LTL potentially reflects decreased senescence or higher replicative potential in pre-cancerous cells,

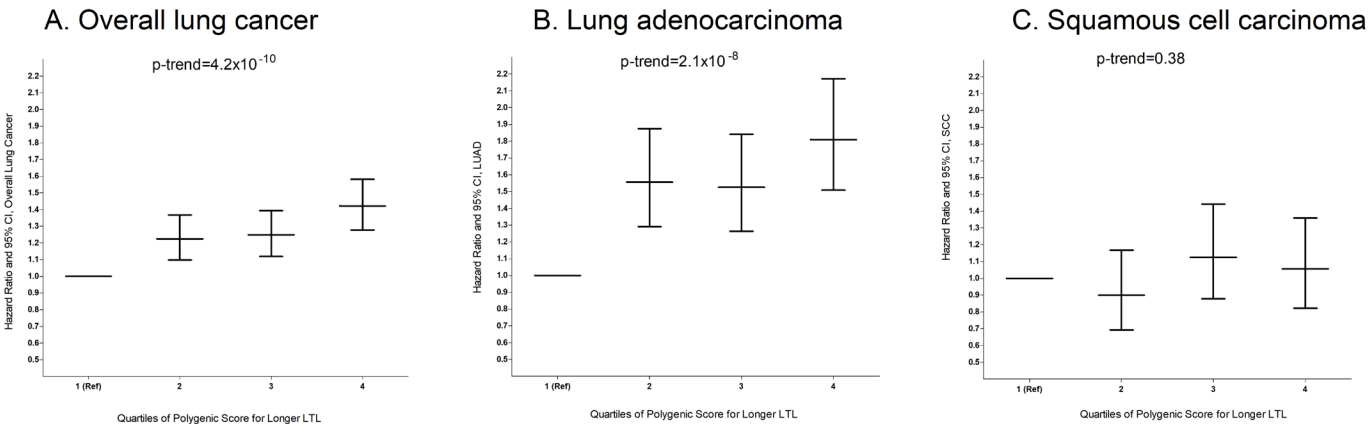

Figure 1 Genetically predicted leucocyte telomere length and risk of lung cancer and its histological subtypes. Using European data from the UK Biobank, we constructed a weighted PGS that predicts longer LTL. This PGS was based on 130 out of 197 genetic variants identified in the UK Biobank that reached genome-wide significance ($p<8.31\times10^{-9}$). These genetic variants were included in the previous MR analyses and were LD pruned ($R^2>0.01$) (Codd et al. *Nat Genet* 2021). The weights were based on the previously reported Z-standardised beta estimates for SNP-LTL associations (Codd et al. *Nat Genet* 2021). We categorised the PGS into quartiles and analysed associations with risk of lung cancer, LUAD, and SCC among Europeans using multivariable Cox regression adjusted for sex, body mass index, age at recruitment, detailed smoking history/intensity, assessment centre, Townsend Deprivation Index, alcohol use, and secondhand smoke exposure. Linear trends were estimated by analysing continuous PGS.

which allows accumulation of genetic alterations that initiate or promote carcinogenesis.

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Contributors JYYW wrote the manuscript, analyzed data, and led the study. BB, AKH, JS, WH, and MLR edited the manuscript and analyzed data. MJM and SMG edited the manuscript. NR and QL edited the manuscript and supervised the study.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by North West - Haydock Research Ethics Committee 3rd Floor - Barlow House 4 Minshull Street Manchester M1 3DZ Telephone: 02071048103. Title of the Database: UK Biobank: a large scale prospective epidemiological resource. Designated Individual: Mrs. Samantha Welsh. REC reference: 21/NW/0157IRAS. project ID: 299116 Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

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Supplementary Materials

Methods

Study Design

Details of the study design of the UK Biobank have been described (http://www.ukbiobank.ac.uk/) [1 2]. Briefly, the target population was adults aged 40-69 years who resided within 40 km of 22 assessment centers situated throughout the UK near roads or transit points. Among the 9.2 million people registered in the UK's National Health Service (NHS) who were mailed invitations to participate in the study, 503,317 people (5.5%) responded and visited the assessment centers between 2006 and 2010 [1]. The study participants were administered touchscreen questionnaires, physical examinations, and provided biological samples. The UK Biobank dataset is continually updated and data from 502,409 participants were available for our analyses as of August 2022 (UK Biobank project number: 28072).

Baseline Exclusion Criteria

Among the 502,409 study participants, we excluded 372 participants with discrepancies between self-reported gender and genetic sex; 46,577 participants who reported any prevalent cancer diagnosis at or before baseline; 57,253 participants who reported respiratory diseases at or before baseline that could potentially influence LTL and future risk of lung cancer (i.e., asthma, chronic obstructive pulmonary disease, emphysema, bronchitis, bronchiectasis, interstitial lung disease, asbestosis, pulmonary fibrosis, or other respiratory problems); and 4,779 participants who reported hematologic/immunologic disorders at or before baseline (i.e., hemophilia, anemia, sickle cell disease, thalassemia, HIV infection/AIDS, clotting/excessive bleeding, neutropenia,

lymphopenia, myeloproliferative disorder, and hereditary/genetic hematological disorder). After these baseline exclusions, our analytic dataset was composed of 393,428 participants.

Study Follow-up

Follow-up time started for each participant at the date of visit to the assessment center and ended at the date of primary incident lung cancer diagnosis (outcome), death (censored), or administrative censoring (i.e., September 20th 2021 for England and Wales and October 31st 2021 for Scotland), whichever came first. Cancer diagnoses were provided to UK Biobank by the Health and Social Care Information Centre (HSCIC) and the NHS Central Register (NHSCR). For our analyses, lung cancer diagnosis was defined by International Classification of Diseases 10th revision (ICD-10 codes) C34.0-C34.9. ICD-O-3 code 8140 was used to define adenocarcinoma, while 8052, 8084, 8073, and 8083 were used for squamous cell carcinoma (SCC). The NHS Information Centre and the NHS Central Register Scotland provided vital status, as well as date and primary underlying cause of death for participants.

The UK Biobank study was approved by the National Information Governance Board for Health and Social Care and the NHS North West Multicenter Research Ethics Committee. Electronic informed consent was obtained from all volunteer participants

Measured phenotypic leukocyte telomere length

Measurement of phenotypic LTL using quantitative polymerase chain reaction (qPCR) along with quality control analyses were performed at the University of Leicester as previously

described in detail [3]. Measurement of LTL was conducted using extracted genomic DNA from peripheral blood leukocytes from a cohort-wide array genotyping project as previously reported [4]. Data from the multiplex qPCR assay were used to generate the ratio of telomere repeat copy number (T) relative to that of a single copy gene (S, Human beta-globin (Hgb)) for each subject [5], which is known as the 'T/S ratio'. The purpose of S was to control for the number of genomes in the input DNA sample in the qPCR reaction. The T/S ratio for each subject was then divided by the T/S ratio of a calibrator sample (pooled DNA from 20 individuals), which was included on every run, to generate the 'relative T/S ratio'. The purpose of the calibrator sample was to account for run-to-run variation. The 'relative T/S' ratio reflects the average telomere abundance across all chromosomes in a population of leukocytes in an individual (i.e., phenotypic LTL).

Questionnaire-based covariates

The touchscreen questionnaire administered at baseline collected information on demographics, lifestyle factors, socioeconomic status, and anthropometric measurements. A detailed 28-category smoking history/intensity variable was created as previously described [6]. A 6-level variable for alcohol intake was generated by combining data on drinking status and intensity (never, former, current infrequent (<3 times per month), current modest (<1 drink per day), current frequent (≥1 to ≤3 drinks per day), and current heavy (>3 drinks per day)). Body mass index (BMI) was categorized as <18.5, ≥18.5 to <25.0, ≥25.0 to <30.0, ≥30.0 to <35.0, and ≥35.0 kg/m^2. Race/ethnicity/ancestry was categorized as European, Asian, Black (African ancestry), mixed, other, and missing/unknown. We used the continuous Townsend Deprivation Index as a proxy for socioeconomic status.

Supplementary Table 1: Sensitivity analyses -Associations between measured phenotypic leukocyte telomere length and lung cancer risk including participants with chronic respiratory disease at study enrollment

Quartile	Adjusted Relative T/S Ratio Cutoffs	No. of incident cases	HR	95% CI Lower	95% CI Upper	p-value	
		I) Overall lung cancer (3377 cases)					
1	<0.74	1043	1.00				
2	0.74 to <0.82	810	0.96	0.87	1.05	0.32	
3	0.82 to <0.91	783	1.06	0.97	1.16	0.22	
4	≥0.91	741	1.23	1.12	1.35	$2.9x10^{-5}$	*
					p-trend	$4.5x10^{-5}$	*
		II) Lung adenocarcinoma (1264 cases)					
		No. of incident cases	**HR**	**95% CI Lower**	**95% CI Upper**	**p-value**	
1	<0.74	321	1.00				
2	0.74 to <0.82	306	1.13	0.97	1.32	0.13	
3	0.82 to <0.91	296	1.23	1.05	1.45	0.01	*
4	≥0.91	341	1.68	1.44	1.97	$4.8x10^{-11}$	*
					p-trend	$9.1x10^{-11}$	*
		III) Lung squamous cell carcinoma (610 cases)					
		No. of incident cases	**HR**	**95% CI Lower**	**95% CI Upper**	**p-value**	
1	<0.74	229	1.00				
2	0.74 to <0.82	151	0.87	0.71	1.07	0.17	
3	0.82 to <0.91	138	0.94	0.76	1.17	0.59	
4	≥0.91	92	0.81	0.63	1.04	0.09	
					p-trend	0.10	

The sensitivity analyses included a total of 425,108 participants, inclusive of 57,253 participants who reported respiratory diseases at or before baseline that could potentially influence LTL and future risk of lung cancer (i.e., asthma, chronic obstructive pulmonary disease, emphysema, bronchitis, bronchiectasis, interstitial lung disease, asbestosis, pulmonary fibrosis, or other respiratory problems). Multivariable Cox regression models were used to estimate hazard ratios (HR) and 95% confidence intervals (CI) of incident lung cancer in relation to quartiles of *adjusted relative* T/S Ratio (i.e., measured phenotypic LTL), adjusted for age at recruitment (continuous), sex (men vs. women), race/ethnicity/ancestry (reference: European), detailed smoking history/intensity (28 categories, reference: never smokers), study assessment center, BMI (reference: ≥18.5 to <25.0 kg/m^2), Townsend Deprivation Index (continuous), alcohol intake (reference: never drinker), exposure to secondhand smoke (ever vs. never), and leukocyte subtype differentials (% lymphocytes, neutrophils, eosinophils, basophils and monocytes). P-trends were estimated using continuous log-transformed *adjusted relative* T/S ratio. *P-values <0.05 were considered statistically significant. Discrepancy in counts was due to missing LTL data.

Supplementary Table 2: Associations between measured phenotypic leukocyte telomere length and risk of lung adenocarcinoma and squamous cell carcinoma in the UK Biobank among subgroups defined by sex and smoking status

Quartile	Adjusted Relative T/S Ratio Cutoffs	No. of incident cases	HR	95% CI Lower	95% CI Upper	p-value	
A) Lung adenocarcinoma							
I) NEVER SMOKERS		200 cases / 205,237 subjects					
1	<0.74	43	1.00				
2	0.74 to <0.82	35	0.85	0.54	1.33	0.47	
3	0.82 to <0.91	47	1.20	0.79	1.82	0.39	
4	≥0.91	67	1.84	1.25	2.72	2.2×10^{-3}	*
					p-trend	8.7×10^{-5}	*
II) FORMER SMOKERS		508 cases / 125,995 subjects					
1	<0.74	116	1.00				
2	0.74 to <0.82	117	1.18	0.91	1.53	0.21	
3	0.82 to <0.91	126	1.44	1.12	1.86	4.9×10^{-3}	*
4	≥0.91	132	1.80	1.39	2.31	6.2×10^{-6}	*
					p-trend	1.1×10^{-5}	*
III) CURRENT SMOKERS							
		359 cases / 38,808 subjects					
1	<0.74	91	1.00				
2	0.74 to <0.82	100	1.40	1.05	1.86	0.02	*
3	0.82 to <0.91	62	0.99	0.72	1.38	0.97	
4	≥0.91	93	1.83	1.36	2.46	6.0×10^{-5}	*
					p-trend	2.1×10^{-3}	*
IV) NEVER SMOKING WOMEN		132 cases / 118,044 subjects					
Quartile	**Adjusted Relative T/S Ratio Cutoffs**	**No. of incident cases**	**HR**	**95% CI Lower**	**95% CI Upper**	**p-value**	
1	<0.74	27	1.00				
2	0.74 to <0.82	20	0.72	0.40	1.28	0.26	
3	0.82 to <0.91	33	1.18	0.71	1.96	0.53	
4	≥0.91	47	1.68	1.04	2.71	0.04	*
					p-trend	4.0×10^{-3}	*
V) NEVER SMOKING MEN		68 cases / 87,193 subjects					
1	<0.74	16	1.00				
2	0.74 to <0.82	15	1.13	0.56	2.29	0.73	

3	0.82 to <0.91	14	1.24	0.60	2.55	0.56	
4	≥0.91	20	2.24	1.15	4.38	0.02	*
					p-trend	$5.1x10^{-3}$	*
VI) FORMER SMOKING WOMEN		256 cases / 59,763 subjects					
1	<0.74	45	1.00				
2	0.74 to <0.82	58	1.31	0.89	1.94	0.17	
3	0.82 to <0.91	71	1.67	1.15	2.42	0.01	*
4	≥0.91	76	1.94	1.34	2.82	$4.7x10^{-4}$	*
					p-trend	$2.3x10^{-4}$	*
VII) FORMER SMOKING MEN		252 cases / 66,232 subjects					
1	<0.74	71	1.00				
2	0.74 to <0.82	59	1.10	0.78	1.55	0.60	
3	0.82 to <0.91	55	1.27	0.89	1.81	0.19	
4	≥0.91	56	1.73	1.21	2.47	$2.4x10^{-3}$	*
					p-trend	$9.6x10^{-3}$	*
VIII) CURRENT SMOKING WOMEN		164 cases/ 16,649 subjects					
1	<0.74	42	1.00				
2	0.74 to <0.82	43	1.12	0.73	1.72	0.60	
3	0.82 to <0.91	29	0.77	0.48	1.23	0.27	
4	≥0.91	44	1.35	0.88	2.07	0.17	
					p-trend	0.56	
IX) CURRENT SMOKING MEN		195 cases / 22,159 subjects					
1	<0.74	49	1.00				
2	0.74 to <0.82	57	1.63	1.11	2.39	0.01	*
3	0.82 to <0.91	33	1.21	0.78	1.89	0.40	
4	≥0.91	49	2.35	1.57	3.52	$3.2x10^{-5}$	*
					p-trend	$3.1x10^{-4}$	*
B) Lung squamous cell carcinoma							
		No. of incident cases	**HR**	**95% CI Lower**	**95% CI Upper**	**p-value**	
I) NEVER SMOKERS							
1	<0.74		Not analyzable				
2	0.74 to <0.82						
3	0.82 to <0.91						

4	≥0.91						
II) FORMER SMOKERS		203 cases / 125,995 subjects					
1	<0.74	71	1.00				
2	0.74 to <0.82	53	0.95	0.67	1.36	0.79	
3	0.82 to <0.91	44	0.94	0.65	1.38	0.77	
4	≥0.91	29	0.80	0.52	1.24	0.31	
					p-trend	0.20	
III) CURRENT SMOKERS							
		251 cases / 38,808 subjects					
1	<0.74	87	1.00				
2	0.74 to <0.82	60	0.96	0.69	1.33	0.79	
3	0.82 to <0.91	58	1.09	0.78	1.53	0.61	
4	≥0.91	36	0.88	0.60	1.31	0.53	
					p-trend	0.70	
IV) NEVER SMOKING WOMEN							
Quartile	**Adjusted Relative T/S Ratio Cutoffs**	**No. of incident cases**	**HR**	**95% CI Lower**	**95% CI Upper**	**p-value**	
1	<0.74		Not analyzable				
2	0.74 to <0.82						
3	0.82 to <0.91						
4	≥0.91						
V) NEVER SMOKING MEN							
1	<0.74		Not analyzable				
2	0.74 to <0.82						
3	0.82 to <0.91						
4	≥0.91						
VI) FORMER SMOKING WOMEN		62 cases / 59,763 subjects					
1	<0.74	22	1.00				
2	0.74 to <0.82	11	0.55	0.26	1.13	0.10	
3	0.82 to <0.91	17	0.92	0.49	1.75	0.80	
4	≥0.91	9	0.56	0.26	1.23	0.15	
					p-trend	0.10	

VII) FORMER SMOKING MEN		141 cases / 66,232 subjects					
1	<0.74	49	1.00				
2	0.74 to <0.82	42	1.18	0.78	1.78	0.44	
3	0.82 to <0.91	27	0.94	0.58	1.50	0.78	
4	≥0.91	20	0.95	0.56	1.60	0.84	
					p-trend	0.68	
VIII) CURRENT SMOKING WOMEN		79 cases / 16,649 subjects					
1	<0.74	33	1.00				
2	0.74 to <0.82	13	0.45	0.24	0.86	0.01 *	
3	0.82 to <0.91	15	0.54	0.29	1.00	0.05	
4	≥0.91	18	0.80	0.44	1.43	0.45	
					p-trend	0.16	
IX) CURRENT SMOKING MEN		172 cases / 22,159 subjects					
1	<0.74	54	1.00				
2	0.74 to <0.82	47	1.28	0.86	1.90	0.22	
3	0.82 to <0.91	43	1.45	0.97	2.18	0.07	
4	≥0.91	18	0.81	0.47	1.39	0.44	
					p-trend	0.78	

Multivariable Cox regression models were used to estimate hazard ratios (HR) and 95% confidence intervals (CI) of incident lung adenocarcinoma or squamous cell carcinoma in relation to quartiles of *adjusted relative* T/S Ratio (i.e., measured phenotypic LTL), adjusted for age at recruitment (continuous), race/ethnicity/ancestry (reference: European), detailed smoking history/intensity when analyzing former and current smokers, study assessment center, BMI (reference: ≥18.5 to <25.0 kg/m^2), Townsend Deprivation Index (continuous), alcohol intake (reference: never drinker), exposure to secondhand smoke (ever vs. never), and leukocyte subtype differentials (% lymphocytes, neutrophils, eosinophils, basophils and monocytes). P-trends were estimated using continuous log-transformed *adjusted relative* T/S ratio. *P-values <0.05 were considered statistically significant. Discrepancy in counts were due to missing LTL data.

References for Supplementary Materials

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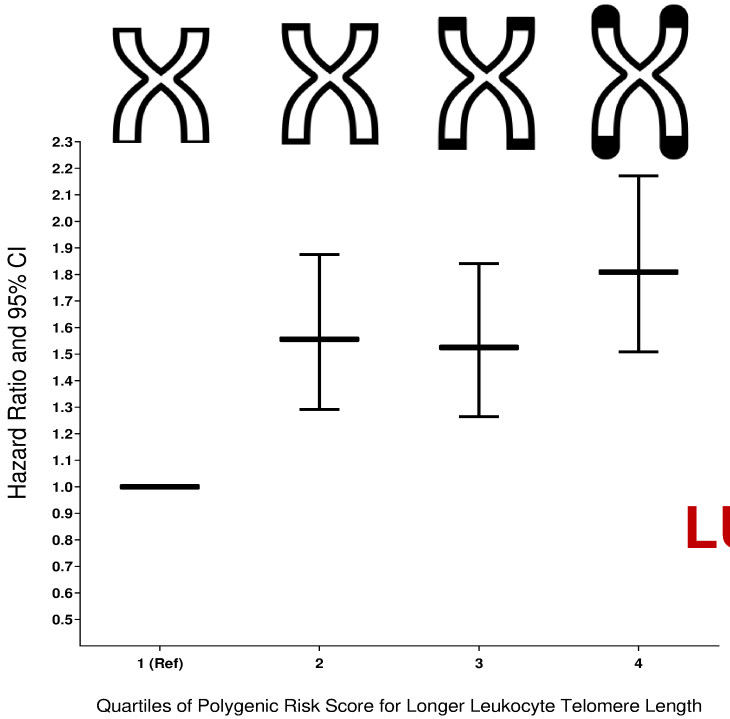

LONGER LEUKOCYTE TELOMERE LENGTH

INCREASED RISK OF LUNG ADENOCARCINOMA