Genetic variants associated with longer telomere length are associated with increased lung cancer risk among never-smoking women in Asia: A report from the Female Lung Cancer Consortium in Asia

A full list of authors and affiliations appears at the end of the article.

Abstract

Recent evidence from several relatively small nested case-control studies in prospective cohorts shows an association between longer telomere length measured phenotypically in peripheral white blood cell (WBC) DNA and increased lung cancer risk. We sought to further explore this relationship by examining a panel of 7 telomere-length associated genetic variants in a large study of 5,457 never-smoking female Asian lung cancer cases and 4,493 never-smoking female Asian controls using data from a previously reported genome-wide association study. Using a group of 1,536 individuals with phenotypically measured telomere length in WBCs in the prospective Shanghai Women's Health study, we demonstrated the utility of a genetic risk score (GRS) of 7 telomere-length associated variants to predict telomere length in an Asian population. We then found that GRSs used as instrumental variables to predict longer telomere length were associated with increased lung cancer risk (OR = 1.51 (95% CI=1.34–1.69) for upper vs. lower quartile of the weighted GRS, P-value=4.54×10^{-14}) even after removing rs2736100 (P-value=4.81×10^{-3}), a SNP in the *TERT* locus robustly associated with lung cancer risk in prior association studies. Stratified analyses suggested the effect of the telomere-associated GRS is strongest among younger individuals. We found no difference in GRS effect between adenocarcinoma and squamous cell subtypes. Our results indicate that a genetic background that favors longer telomere length may increase lung cancer risk, which is consistent with earlier prospective studies relating longer telomere length with increased lung cancer risk.

Keywords

association study; genetics; lung cancer; telomere length; genetic risk score

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CORRESPONDING AUTHOR: Mitchell J. Machiela, Advanced Technology Center, Rm 225, 8717 Grovemont Circle, Bethesda, MD 20892, office: 301-435-6391; fax: 301-402-3134.
**These authors contributed equally to this work.
*These authors jointly directed this work.

INTRODUCTION

Telomeres are specialized chromatin structures that shorten during each round of cellular division in mammalian cells. Prolonged erosion of telomere length can lead to genetic instability, cellular senescence, and apoptosis[1]. Earlier studies, mainly retrospective, on peripheral WBCs have suggested increased cancer risk associated with shorter telomere length[2–6]. These studies may suffer from disease bias in which telomere shortening was a consequence of tumor growth and progression rather than a risk factor for tumorigenesis. Recent, primarily prospective studies indicate that, contrary to expectation, longer telomere length may be associated with cancer risk[7–14], particularly for lung cancer[15–18].

Telomere length has historically been measured in peripheral WBC by multiplex quantitative polymerase chain reaction[19]. A recent genome-wide association study (GWAS) on telomere length has identified 7 loci robustly associated with WBC telomere length[20]. While genetic variants at these loci explain a small proportion of the total biological variation in telomere length, the age-related shortening per variant risk allele was equivalent to 1.9–3.9 years of attrition in telomere/single copy gene (T/S) ratio, equating to approximately 57 to 117 base pairs in telomere length per risk allele. Furthermore, the authors demonstrated the utility of genetic risk scores (GRS) of these variants to replicate a well-established association between shorter mean peripheral WBC telomere length and coronary artery disease. This suggests that by using telomere-length associated GRS as an instrument to approximate telomere shortening or lengthening, causal relationships with telomere length can be investigated in etiologically complex diseases that include environmental risk factors associated with both disease risk and telomere length.

We herein report an investigation of the 7 identified telomere-length associated variants in a sample of lung cancer cases and controls from a population of never-smoking Asian females. Our investigation uses data generated as part of a previously reported genome-wide association study (GWAS) conducted by the Female Lung Cancer Consortium in Asia[21]. Our objectives are to (1) validate the utility of these 7 telomere-length associated variants discovered in a primarily European population to predict measured telomere length in an Asian population; (2) characterize overall and individual associations of telomere-length associated variants with lung cancer risk; (3) investigate the ability of GRSs of these variants to predict lung cancer risk; and (4) describe the direction of the associations observed between telomere-length associated variants and lung cancer risk.

MATERIAL AND METHODS

Study subjects were from a published GWAS investigating lung cancer susceptibility risk in female Asian non-smokers drawn from 14 studies from mainland China, South Korea, Japan, Singapore, Taiwan, and Hong Kong[21]. Cases had histologically confirmed lung cancer. Each study was approved by the Institutional Review Board of the investigator's institution, and all participants provided written informed consent.

Genotyping was performed in the Cancer Genomics Research Laboratory of the National Cancer Institute's Division of Cancer Epidemiology and Genetics (Gaithersburg, MD);

Gene-Square Biotech, Inc. (Beijing, China); GeneTech Biotech Co. (Taiwan); deCODE Genetics (Iceland); Memorial Sloan-Kettering Cancer Center (New York, NY); and Genome Institute of Singapore (Singapore). Genotyping was carried out on commercially available Illumina Infinium BeadArray human assays (Illumina 370k, Illumina 610Q, and Illumina 660W SNP microarrays) following standard procedures. The methods and quality control metrics applied to genotyping with SNP microarrays have been previously published[21]. Briefly, samples were excluded with low completion rates, extreme heterozygosity values, gender discordance, low Asian ancestry (less than 86%), and first degree relatives were removed. After quality control filtering, a total of 5,510 cases and 4,544 controls had genetic data available for analysis.

To address potential population substructure, principal components were calculated using the GLU struct.pca module (http://code.google.com/p/glu-genetics/) using 33,165 SNPs with low pairwise correlation ($R^2 < 0.01$).

Genotype imputation was performed to ensure complete data existed for all 7 telomere-length associated variants. The IMPUTE2 program (http://mathgen.stats.ox.ac.uk/impute/impute_v2.html) was used with the March 2012 release of the 1,000 Genomes Project data[22] and the DCEG Imputation Reference Set [23] as merged references for imputation. The DCEG reference set serves as a supplement to the 1,000 Genomes reference and includes 2.8 million autosomal polymorphic SNPs for 1,249 individuals, of which 162 individuals are of Asian ancestry. Since the genotyping data was on NCBI Build 36, all genotyped variant coordinates were converted to NCBI Build 37 using UCSC's liftOver utility (http://hgdownload.cse.ucsc.edu/downloads.html) before performing genotype imputation. Recommended IMPUTE2 default settings were used and all imputed SNPs (rs7675998, rs8105767, rs755017, rs11125529) achieved INFO scores >0.99. There was no evidence for significant departures from Hardy-Weinberg proportions (P-value>0.05).

A group of subjects included in previous nested case-control studies of various cancers in the prospective Shanghai Women's Health Study (N=1,536) had both genotyping data and experimentally measured peripheral WBC telomere length that we used to validate the telomere-length associated variants in an Asian population. Multiplex quantitative polymerase chain reactions were used to quantify telomere length. T/S values were extracted for the analysis and log transformed to improve normality.

All plotting and statistical analyses were performed on a 64-bit Windows build of R version 3.0.1 "Good Sport"[24]. Only subjects with complete genotyping, histology, and covariate information were included in the analysis (5,457 cases and 4,493 controls). Models investigating lung cancer risk were adjusted for study indicator variable, 10-year age group indicator variables (<40, 40–49, 50–59, 60–69, and 70+), and significant principal components (EV1, EV2, and EV4), unless otherwise noted. Likelihood-ratio and SNP-set kernel association test (SKAT) linear kernel tests[25, 26] were used to assess statistical significance of aggregations of telomere-length associated variants on lung cancer risk by comparing null models to fitted models containing combinations of the 7 telomere-length associated variants. The SKAT linear kernel test aggregates a set of SNP score test statistics and efficiently computes an overall p-value[26].

Both unweighted and weighted genetic risk scores (GRS) were calculated for telomere-length associated variants. To calculate GRS for the i-th subject from the 7 telomere-length associated variants the following formula was used:

$$GRS_i = \sum_{j=1}^{7} w_j x_{ij}$$

Here, x_{ij} is the number of risk alleles for the j-th SNP in the i-th subject (x_{ij}=0, 1 or 2) and w_j is the weight or coefficient for the j-th SNP. Unweighted genetic risk scores simply counted the number of alleles associated with longer telomere length an individual carried across all 7 telomere-length associated variants, thus giving an equal weight to all risk alleles (w_j=1). Weighted genetic risk scores were calculated likewise, with the addition of assigning previously published telomere-length associated beta estimates[20] as w_j for each telomere-length associated SNP allele count. Weighting normally results in more specificity of the GRS by assigning more weight to variants with stronger effects.

RESULTS

Our dataset consisted of a sample of 5,457 lung cancer cases and 4,493 controls from a population of never-smoking Asian females (Table 1). The participants were drawn from 14 contributing studies with collection areas in mainland China, South Korea, Japan, Singapore, Taiwan, and Hong Kong. Age, a major factor associated with telomere attrition, was available in 10-year age-groups for all participants. Most participants were between 50 and 70 years of age (63%) with 6% of subjects younger than 40 years of age.

Measured and imputed genotypes were available for the 7 telomere-length associated variants (Table 2). Alleles associated with longer telomere length were denoted the risk allele and risk allele frequencies from our dataset were compared to those previously reported by Codd et al[20]. Risk allele frequency differences between our Asian lung cancer study and the Codd et al. study of a population of primarily European descent likely reflect differences in ancestral allele frequencies.

To ensure the telomere-length associated variants, discovered in a population of primarily European ancestry, were a valid surrogate for telomere length in our Asian population, we carried out an analysis on a set of 1,536 Asian females with both measured telomere length and genotype data from the prospective Shanghai Women's Health Study. When testing for an association of each of the 7 telomere length associated variants with measured telomere length, only the *TERT* variant (rs2736100) had a significant association with measured telomere length (P-value=0.03); however, our sample size was substantially smaller than the Codd et al analysis (N=48,423), and although insignificant, 6 of the 7 variants had beta estimates in the correct direction. A weighted GRS with all 7 telomere-length associated variants was calculated and the association with telomere length was also investigated. In the overall sample, the telomere-length associated GRS was significantly associated with measured telomere length (P-value=0.001, Figure 1A), the estimated effect was in the positive direction (beta=0.15), and explained the same percent of total telomere length

variance as in Codd et al.(R^2=0.01)[20]. For the cancer cases in this sample, the mean time between blood sample collection and cancer diagnosis was 5.34 years with 75 percent of cases having blood collected more than 3 years prior to cancer diagnosis. When restricting the analysis to controls (N=533), the association remained significant (P-value=0.04) with similar effect size and variance explained (Figure 1B). Together, this provides evidence the weighted GRS of telomere-length associated variants has utility in predicting measured telomere length in Asian populations.

Overall association tests were conducted to investigate if, in aggregate, all 7 telomere-length associated variants were associated with lung cancer risk. A likelihood ratio test comparing a null model adjusting for 10-year age group, contributing study, and significant principal components to the same model plus all 7 telomere-length associated variants indicated that in aggregate the telomere-length associated variants were significantly associated with lung cancer risk (P-value=9.64×10^{-25}). Furthermore, a linear SKAT found a highly significant association between the 7 telomere-length associated variants and lung cancer (P-value=3.19×10^{-27}).

Each telomere-length associated variant from Codd et al.[20] was tested for an individual association with lung cancer risk. All 7 telomere-length associated variants were included in the same logistic regression model and covariates were included to adjust for 10-year age-group, contributing study, and significant principal components. Two of the 7 telomere-length associated variants (rs2736100 and rs10936599) exhibited association p-values less than 0.05, significantly more than the 0.4 variants expected by chance (P-value=0.04) (Table 2). The rs2736100 variant, located in the first intron of the *TERT* gene, has previously been associated by GWAS with lung cancer risk [27]. Interestingly, 5 of the 7 telomere-length associated variants show effects in the same direction for both the Codd et al. telomere-length association[20] and lung cancer association suggesting enrichment for variants that are associated with both longer telomere length and increased lung cancer risk (Table 2).

Both unweighted and weighted GRSs were calculated as measures of predicted telomere length for each study participant and association with lung cancer risk was tested by logistic regression models that adjusted for 10-year age group, contributing study, and significant principal components. The unweighted telomere-length associated GRS was significantly associated with lung cancer risk (P-value=1.90×10^{-12}), indicating scores associated with longer telomere length were also associated with increased lung cancer risk. The odds ratio comparing individuals in the upper quartile of GRS to those in the lower quartile of GRS was 1.47 (95% CI=1.31–1.65). The beta weighted telomere-length associated GRS demonstrated greater specificity for the lung cancer association with greater evidence for association between longer telomere length and lung cancer risk (P-value=4.54×10^{-14}). A higher odds ratio of 1.51 (95% CI=1.34–1.69) was observed for individuals in the upper quartile of the weighted GRS compared to those in the lower quartile. The association of the weighted GRS across contributing study was homogeneous (homogeneity P-value=0.34) and produced an overall meta-analysis odds ratio of 1.51 (95% CI=1.34–1.71, P-value=1.53×10^{-11}) comparing individuals in the upper quartile of weighted GRS to those in the lower quartile of GRS (Figure 2). When investigating deciles of the weighted GRS, the effect of weighted GRS on lung cancer risk appeared to be monotonic with no threshold

indicating a substantial change in risk (Figure 3). Furthermore, to assess if rs2736100 was the only SNP accounting for the association between the weighted GRS and lung cancer risk, the weighted GRS was recomputed with the exclusion of rs2736100, and rs2736100 used as a separate covariate in the regression model. The weighted GRS minus rs2736100 remained significantly associated with increased lung cancer risk, although the P-value was greatly attenuated (P-value=4.81×10^{-3}).

Additional age-stratified analyses were conducted to investigate potential differences in the weighted GRS lung cancer association with age. Results indicate women in the younger than 60 years age group had an odds ratio of 1.72 (95% CI=1.46–2.02, P-value=9.35×10^{-11}) comparing women in the fourth and first quartiles of weighted GRS, whereas women in the 60 years or older age group had an odds ratio of 1.33 (95% CI=1.12–1.57, P-value=0.001). A significant difference was observed between the two effect estimates (P-value=0.03) indicating the association between weighted telomere-associated GRS and lung cancer risk may be stronger in younger women. Analyses were also stratified based on the two primary histological subtypes of lung cancer: adenocarcinoma and squamous cell carcinoma. The weighted GRS odds ratio comparing the fourth to first quartile for adenocarcinoma cases was 1.51 (95% CI=1.33–1.72, P-value=2.82×10^{-10}). The squamous cell carcinoma odds ratio estimate was slightly lower at 1.42 (95% CI=1.10–1.81, P-value=0.006). A case-only analysis of the two histological subtypes found no significant difference in weighted GRS effect (P-value= 0.80).

DISCUSSION

Our study investigated the relationship between 7 telomere-length associated variants and lung cancer risk. Aggregations of the 7 variants were highly associated with lung cancer risk with the direction of the associations indicating that longer telomere length, as predicted by higher telomere length associated GRS, is a risk factor for lung cancer. Although the telomere-length associated variants explained only a fraction of the variation in telomere length, the associations suggest genetic effects tagged by these variants are important for lung cancer risk.

Previous studies have demonstrated an association with the *TERT* locus (rs2736100)[21, 27] and lung cancer risk, however, our study is the first to provide evidence for associations with other telomere-length associated variants. In particular, the nominal significance of the *TERC* locus (rs10936599) suggests this locus may play a role in telomere-related maintenance important for lung cancer risk, although further studies are needed to verify this association. The 7 telomere-length associated variants explain a limited amount of the total variation in telomere length, suggesting that additional variation in telomere length may be attributable to other genetic variants which remain to be discovered. Additionally, the lower association P-values of aggregate association tests relative to the telomere-length specific GRS tests suggests that in addition to telomere length other aspects of telomeres, such as maintenance of genome stability or chromosomal repair, or distinct biological process tagged by these telomere-length associated variants, especially rs2736100 in *TERT*, may be important contributors to the lung cancer risk.

Using telomere-length associated genetic variants as an instrument for measuring telomere length provides several advantages. First, reverse causation biases that may influence case-control studies of telomere length and disease can be eliminated since telomere-length associated variants are unrelated to time of blood draw and disease diagnosis. Also, by using a correlated genetic proxy for telomere length, it may be possible to partition genetic versus other risk factors (e.g., aging, oxidative damage) that are reflected in the telomere length phenotype. One potential confounder in our analysis is correlated population specific differences between lung cancer frequency and telomere risk allele frequencies. However, this potential population stratification bias was mitigated by adjusting for all principal components that were significantly associated with lung cancer risk.

The biological mechanism linking longer telomere length to lung carcinogenesis is unclear. While telomere attrition leads to replicative senescence and apoptosis, telomere elongation may result in immortalized cells with unregulated telomerase activity and unlimited potential for cellular and tumor growth[28–31]. Shorter telomeres may act as tumor suppressors, whereas longer telomeres may not. In addition, recent evidence suggests excessively long telomeres may be as important for chromosomal instability as critically short telomeres[32].

Our results, as well as an example from coronary artery disease[20], suggest the 7 telomere-associated variants are useful proxies for investigating telomere length in a variety of diseases. Although the 7 variants explain a small portion of measured peripheral WBC telomere length, the age-related shortening per variant risk allele (1.9–3.9 years) and equivalent changes in telomere base pair length (57 to 117 bases) appear to be biologically meaningful for disease risk[20]. Evidence from our analysis suggests that these 7 telomere-associated variants, discovered in a European population, also have application to Asian populations. Additionally, the effect of the weighted GRS appears stronger in younger individuals suggesting telomere-length associated GRSs may be more useful in younger populations with fewer accumulated environmental exposures affecting telomere length than in older populations.

Results from our study indicate the variation tagged by 7 telomere-length associated variants is important for lung cancer risk. Our genetic-based proxy for telomere length suggests longer telomere length is associated with increased lung cancer risk in non-smoking Asian females which is consistent with evidence from a number of relatively small prospective studies of measured telomere length and lung cancer risk with non-smoking cases in Asia and mostly ever-smoking cases of European descent[17]. Further studies investigating the biological mechanisms related to the variation in telomere length captured by these genetic variants will improve understanding of the molecular pathways linking telomere length to lung cancer risk and may elucidate important preventative and therapeutic targets.

Authors

Mitchell J Machiela[1,*], Chao Agnes Hsiung[2,**], Xiao-Ou Shu[3,4,**], Wei Jie Seow[1,**], Zhaoming Wang[5,**], Keitaro Matsuo[6,**], Yun-Chul Hong[7,**], Adeline Seow[8,**], Chen Wu[9,10,**], H Dean Hosgood III[11,**], Kexin Chen[12,**], Jiu-Cun Wang[13,14,**], Wanqing

Wen[3,4], Richard Cawthon[15], Nilanjan Chatterjee[1], Wei Hu[1], Neil E Caporaso[1], Jae Yong Park[16], Chien-Jen Chen[17], Yeul Hong Kim[18], Young Tae Kim[19], Maria Teresa Landi[1], Hongbing Shen[20,21], Charles Lawrence[22], Laurie Burdett[5], Meredith Yeager[5], I-Shou Chang[23], Tetsuya Mitsudomi[24], Hee Nam Kim[25], Gee-Chen Chang[26,27], Bryan A Bassig[1,28], Margaret Tucker[1], Fusheng Wei[29], Zhihua Yin[30], She-Juan An[31], Biyun Qian[32], Victor Ho Fun Lee[33], Daru Lu[13], Jianjun Liu[34,35], Hyo-Sung Jeon[36], Chin-Fu Hsiao[2,37], Jae Sook Sung[38], Jin Hee Kim[39], Yu-Tang Gao[40], Ying-Huang Tsai[41], Yoo Jin Jung[19], Huan Guo[42], Zhibin Hu[20,21], Amy Hutchinson[5], Wen-Chang Wang[2], Robert J Klein[43], Charles C Chung[1], In-Jae Oh[44,45], Kuan-Yu Chen[46], Sonja I Berndt[1], Wei Wu[30], Jiang Chang[9], Xu-Chao Zhang[31], Ming-Shyan Huang[47], Hong Zheng[12], Junwen Wang[48,49], Xueying Zhao[13], Yuqing Li[50], Jin Eun Choi[51], Wu-Chou Su[52], Kyong Hwa Park[18], Sook Whan Sung[53], Yuh-Min Chen[54,55], Li Liu[56], Chang Hyun Kang[19], Lingmin Hu[20,21], Chung-Hsing Chen[23], William Pao[57], Young-Chul Kim[44,45], Tsung-Ying Yang[27], Jun Xu[58], Peng Guan[30], Wen Tan[9], Jian Su[31], Chih-Liang Wang[59], Haixin Li[12], Alan Dart Loon Sihoe[60], Zhenhong Zhao[13], Ying Chen[8], Yi Young Choi[51], Jen-Yu Hung[47], Jun Suk Kim[61], Ho-Il Yoon[62], Qiuyin Cai[3,4], Chien-Chung Lin[52], In Kyu Park[19], Ping Xu[63], Jing Dong[20,21], Christopher Kim[1], Qincheng He[30], Reury-Perng Perng[54], Takashi Kohno[64], Sun-Seog Kweon[65,66], Chih-Yi Chen[67], Roel C H Vermeulen[68], Junjie Wu[13], Wei-Yen Lim[8], Kun-Chieh Chen[27], Wong-Ho Chow[1], Bu-Tian Ji[1], John K C Chan[69], Minjie Chu[20,21], Yao-Jen Li[17], Jun Yokota[70], Jihua Li[71], Hongyan Chen[13], Yong-Bing Xiang[72], Chong-Jen Yu[46], Hideo Kunitoh[73], Guoping Wu[29], Li Jin[13], Yen-Li Lo[2], Kouya Shiraishi[64], Ying-Hsiang Chen[2], Hsien-Chih Lin[2], Tangchun Wu[42,*], Maria Pik Wong[74,*], Yi-Long Wu[31,*], Pan-Chyr Yang[75,*], Baosen Zhou[30,*], Min-Ho Shin[66,*], Joseph F Fraumeni Jr[1,*], Wei Zheng[3,4,*], Dongxin Lin[9,*], Stephen J Chanock[1,*], Nathaniel Rothman[1,*], and Qing Lan[1,*]

Affiliations

[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland, USA [2]Institute of Population Health Sciences, National Health Research Institutes, Zhunan, Taiwan [3]Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee, USA [4]Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, Tennessee, USA [5]Cancer Genomics Research Laboratory, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland, USA [6]Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan [7]Department of Preventive Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea [8]Saw Swee Hock School of Public Health, National University of Singapore, Republic of Singapore [9]Department of Etiology and Carcinogenesis, Cancer Institute and Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China [10]State Key Laboratory of Molecular Oncology, Cancer Institute and Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China [11]Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, New York, USA

[12]Department of Epidemiology and Biostatistics, Key Laboratory of Cancer Prevention and Therapy, National Clinical Research Center of Cancer, Medical University Cancer Institute and Hospital, Tianjin, China [13]Ministry of Education Key Laboratory of Contemporary Anthropology, School of Life Sciences, Fudan University, Shanghai, China [14]State Key Laboratory of Genetic Engineering, School of Life Sciences, Fudan University, Shanghai, China [15]Department of Human Genetics, University of Utah, Salt Lake City, Utah, USA [16]Lung Cancer Center, Kyungpook National University Medical Center, Daegu, Republic of Korea [17]Genomic Research Center, Taipei, Taiwan [18]Department of Internal Medicine, Division of Oncology/Hematology, College of Medicine, Korea University Anam Hospital, Seoul, Republic of Korea [19]Department of Thoracic and Cardiovascular Surgery, Cancer Research Institute, Seoul National University College of Medicine, Seoul, Republic of Korea [20]Jiangsu Collaborative Innovation Center For Cancer Personalized Medicine, Nanjing Medical University, Nanjing, China [21]Jiangsu Key Laboratory of Cancer Biomarkers, Prevention and Treatment, Cancer Center, School of Public Health, Nanjing Medical University, Nanjing, China [22]Westat, Rockville, Maryland, USA [23]National Institute of Cancer Research, National Health Research Institutes, Zhunan, Taiwan [24]Division of Thoracic Surgery, Kinki University School of Medicine, Sayama, Japan [25]Center for Creative Biomedical Scientists, Chonnam National University, Gwangju, Republic of Korea [26]Department of Medicine, School of Medicine, National Yang-Ming University, Taipei, Taiwan [27]Department of Internal Medicine, Division of Chest Medicine, Taichung Veterans General Hospital, Taichung, Taiwan [28]Division of Environmental Health Sciences, Yale School of Public Health, New Haven, Connecticut, USA [29]China National Environmental Monitoring Center, Beijing, China [30]Department of Epidemiology, School of Public Health, China Medical University, Shenyang, China [31]Guangdong Lung Cancer Institute, Guangdong Provincial Key Laboratory of Translational Medicine in Lung Cancer, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, China [32]Department of Epidemiology and Biostatistics, School of Public Health, Shanghai Jiao Tong University, Shanghai, China [33]Department of Clinical Oncology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China [34]Department of Human Genetics, Genome Institute of Singapore, Republic of Singapore [35]School of Life Sciences, Anhui Medical University, Hefei, China [36]Molecular Diagnostics and Imaging Center, Kyungpook National University, Daegu, Republic of Korea [37]Taiwan Lung Cancer Tissue/Specimen Information Resource Center, National Health Research Institutes, Zhunan, Taiwan [38]Cancer Research Institute, Korea University, Seoul, Republic of Korea [39]Department of Environmental Health, Graduate School of Public Health, Seoul National University, Seoul, Republic of Korea [40]Department of Epidemiology, Shanghai Cancer Institute, Shanghai, China [41]Department of Respiratory Thearpy, Chang Gung Memorial Hospital, Chiayi, Taiwan [42]Institute of Occupational Medicine and Ministry of Education Key Lab for Environment and Health, School of Public Health, Huazhong University of Science and Technology, Wuhan, China [43]Icahn Institute for Genomics and Multiscale Biology, Department of

Author Manuscript

Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, New York, USA [44]Lung and Esophageal Cancer Clinic, Chonnam National University Hwasun Hospital, Hwasun-eup, Republic of Korea [45]Department of Internal Medicine, Chonnam National University Medical School, Gwangju, Republic of Korea [46]Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan [47]Department of Internal Medicine, Kaohsiung Medical University Hospital, School of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan [48]Centre for Genomic Sciences, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China [49]Department of Biochemistry, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China [50]Cancer Prevention Institute of California, Fremont, California, USA [51]Cancer Research Center, Kyungpook National University Medical Center, Daegu, Republic of Korea [52]Department of Internal Medicine, Cancer Center, National Cheng Kung University Hospital, National College of Medicine, Cheng Kung University, Tainan, Taiwan [53]Department of Thoracic and Cardiovascular Surgery, Seoul St Mary's Hospital, Seoul, Republic of Korea [54]Department of Chest Medicine, Taipei Veterans General Hospital, Taipei, Taiwan [55]Taipei Cancer Center, Taipei Medical University, Taipei, Taiwan [56]Cancer Center, Union Hospital, Huazhong University of Science and Technology, Wuhan, China [57]Division of Hematology and Oncology, Vanderbilt University Medical Center, Nashville, Tennessee, USA [58]School of Public Health, The University of Hong Kong, Hong Kong [59]Department of Pulmonary and Critical Care, Chang Gung Memorial Hospital, Taoyuan, Taiwan [60]Department of Surgery, Division of Cardiothoracic Surgery, Queen Mary Hospital, Hong Kong, China [61]Department of Internal Medicine, Division of Medical Oncology, College of Medicine, Korea University Guro Hospital, Seoul, Republic of Korea [62]Department of Internal Medicine, Seoul National University Bundang Hospital, Seongnam, Republic of Korea [63]Department of Oncology, Wuhan Iron and Steel Corporation Staff Worker Hospital, Wuhan, China [64]Division of Genome Biology, National Cancer Center Research Institute, Tokyo, Japan [65]Jeonnam Regional Cancer Center, Chonnam National University Hwasun Hospital, Hwasun-eup, Republic of Korea [66]Department of Preventive Medicine, Chonnam National University Medical School, Gwangju, Republic of Korea [67]Institute of Medicine, Division of Thoracic Surgery, Department of Surgery, Chung Shan Medical University, Chung Shan Medical University Hospital, Taichung, Taiwan [68]Division of Environmental Epidemiology, Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, The Netherlands [69]Department of Pathology, Queen Elizabeth Hospital, Hong Kong, China [70]Institute of Predictive and Personalized Medicine of Cancer (IMPPC), Barcelona, Spain [71]Qujing Center for Diseases Control and Prevention, Sanjiangdadao, Qujing, China [72]Shanghai Cancer Institute, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China [73]Department of Medical Oncology, Japanese Red Cross Medical Center, Tokyo, Japan [74]Department of Pathology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China [75]Department of Internal Medicine, National Taiwan University College of Medicine, Taipei, Taiwan

Acknowledgments

ABBREVIATIONS

DNA	deoxyribonucleic acid
GRS	genetic risk score
GWAS	genome-wide association study
SKAT	snp-set kernel association test

SNP	single nucleotide polymorphism
T/S	telomere/single copy gene
WBC	white blood cell

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NOVELTY AND IMPACT STATEMENT

Using 7 telomere-length associated SNPs to estimate telomere length, we observe a positive association between longer telomere length and increased lung cancer risk in female Asian never smokers. Our genetic proxy is not affected by reverse-causation bias or environmental exposures and supports prior evidence from smaller prospective studies. The variation in telomere length captured by these variants may aid in linking specific biological mechanisms related to telomere length with lung cancer risk.
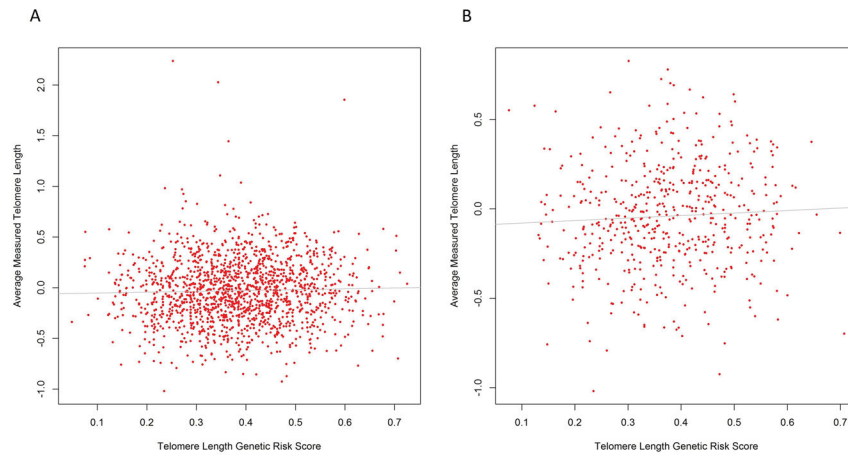
FIGURE 1. Relation of telomere-length associated variants with measured telomere length in peripheral white blood cell DNA from 1,536 women included in previous nested case-control studies of various cancers in the Shanghai Women's Health Study

A best-fit line (solid gray line) is drawn for the relationship of measured log-transformed telomere length with telomere-length associated weighted genetic risk score for (A) cancer cases and controls (R^2=0.01, P-value=0.001) and (B) controls (N = 533) only (R^2=0.01, P-value=0.04).

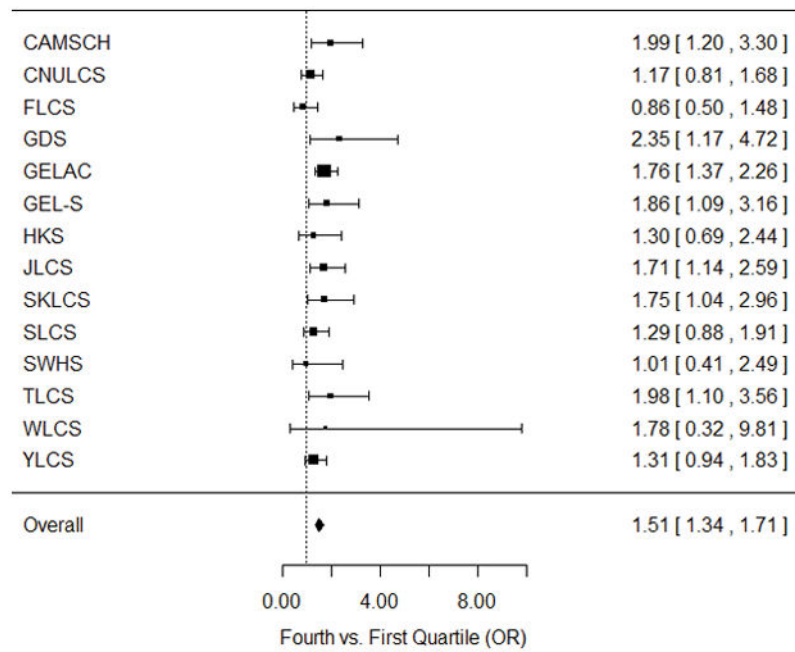**FIGURE 2. Adjusted odds ratios for risk of lung cancer among never-smoking females in Asia comparing upper quartile to lower quartile of weighted telomere length genetic risk scores, by study**

Lung cancer risk was positively associated with increasing weighted GRS (P-value=1.53×10^{-11}) with no significant evidence for heterogeneity of effect (P-value=0.34).
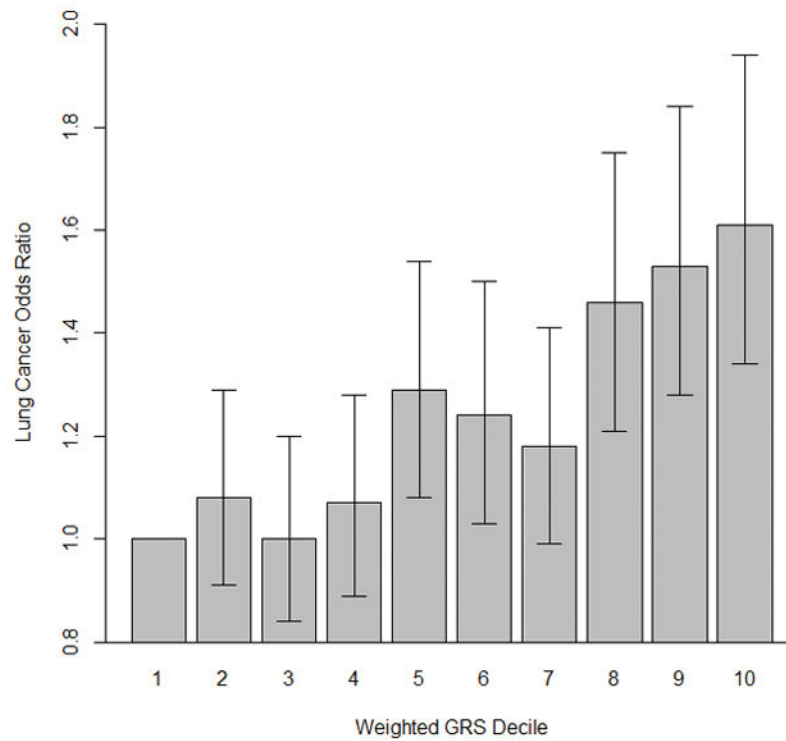
FIGURE 3. Adjusted odds ratios of weighted telomere length genetic risk scores with lung cancer risk among never-smoking females in Asia, by decile

Bars are lung cancer association odds ratios for each weighted GRS decile and error bars represent 95% confidence intervals around the odds ratios. The first decile is used as the reference group with an odds ratio of one. As compared to individuals with a telomere-length associated GRS in the first decile, individuals with a GRS in the tenth decile have a 61% (95% CI=34–94%, P-value=2.83×10^{-7}) increased odds of developing lung cancer.

TABLE 1

Age distribution, by study, of lung cancer cases and controls among never-smoking females in Asia

Study Group*	Region	Asian Lung Cancer Study			Age Group Distribution				
		N	Cases	Controls	39	40–49	50–59	60–69	70+
CAMSCH	Mainland China	744	541	203	8%	21%	33%	28%	10%
CNULCS	South Korea	1036	585	451	4%	9%	30%	35%	22%
FLCS	Mainland China	522	196	326	2%	7%	39%	38%	14%
GDS	Mainland China	648	533	115	14%	19%	27%	25%	13%
GELAC	Taiwan	2043	1065	978	3%	13%	31%	33%	21%
GEL-S	Singapore	483	187	296	2%	9%	26%	27%	36%
HKS	Hong Kong	342	132	210	6%	13%	23%	29%	29%
JLCS	Japan	886	405	481	5%	10%	28%	51%	7%
SKLCS	South Korea	557	334	223	3%	11%	23%	41%	22%
SLCS	Mainland China	951	556	395	10%	18%	27%	34%	11%
SWHS	Mainland China	161	88	73	0%	17%	35%	47%	1%
TLCS	Mainland China	438	299	139	4%	18%	41%	27%	9%
WLCS	Mainland China	61	30	31	3%	30%	38%	20%	10%
YLCS	Mainland China	1078	506	572	9%	24%	33%	23%	10%
		9950	5457	4493	6%	15%	30%	33%	16%

*
CAMSCH: Chinese Academy of Medical Sciences Cancer Hospital Study; CNULCS: Chonnam National University Lung Cancer Study; FLCS: Fudan Lung Cancer Study; GDS: Guangdong Study; GELAC: Genetic Epidemiological Study of Lung Adenocarcinoma (in Taiwan); GEL-S: Genes and Environment in Lung Cancer, Singapore study; HKS: Hong Kong Study; JLCS: Japanese Female Lung Cancer Collaborative Study; SKLCS: South Korea Multi-Center Lung Cancer Study (includes Seoul National University Study; Korean University Medical Center Study; and Kyungpook National University Hospital Study); SLCS: Shenyang Lung Cancer Study; SWHS: Shanghai Women's Health Study; TLCS: Tianjin Lung Cancer Study; WLCS: Wuhan Lung Cancer Study; YLCS: Yunnan Lung Cancer Study.

TABLE 2

Relationship between genetic risk alleles, previously reported measured telomere length in peripheral white blood cells from a large study in a European population, and risk of lung cancer among never-smoking females in Asia

SNP	Chr	Position (GRCh37/hg19)	Nearby Gene	Alleles		Genetic Variants and Telomere Length in a European Population (Codd et al.)[†]				Genetic Variants and Lung Cancer Association in a Female Non-smoking Asian Population				
				Risk*	Non-Risk	Telomere Length Association				RAF		Lung Cancer Association[‡]		
						RAF Controls	β	95% CI	P-value	Controls	Cases	OR	95% CI	P-value
rs10936599	3	169,492,101	TERC	C	T	0.748	0.097	(0.081–0.113)	2.54×10^{-31}	0.431	0.451	1.08	(1.01–1.14)	0.014
rs2736100	5	1,286,516	TERT	C	A	0.486	0.078	(0.060–0.096)	4.38×10^{-19}	0.397	0.480	1.38	(1.30–1.47)	1.09×10^{-26}
rs7675998	4	164,007,820	NAF1	G	A	0.783	0.074	(0.056–0.092)	4.35×10^{-16}	0.826	0.833	1.05	(0.97–1.14)	0.202
rs9420907	10	105,676,465	OBFC1	C	A	0.135	0.069	(0.049–0.089)	6.90×10^{-11}	0.009	0.009	0.92	(0.67–1.26)	0.603
rs8105767	19	22,215,441	ZNF208	G	A	0.291	0.048	(0.032–0.064)	1.11×10^{-9}	0.318	0.326	1.04	(0.98–1.11)	0.191
rs755017	20	62,421,622	RTEL1	G	A	0.131	0.062	(0.040–0.084)	6.71×10^{-9}	0.447	0.443	0.98	(0.93–1.04)	0.536
rs11125529	2	54,475,866	ACYP2	A	C	0.142	0.056	(0.036–0.076)	4.48×10^{-8}	0.166	0.178	1.07	(1.00–1.16)	0.066

*
Risk allele is the allele associated with longer telomere length.

[†]
Data extracted from Table 1 of the Codd et al. [20].

[‡]
Association adjusted for age group, contributing study, and significant eigenvectors.

Abbreviations: chromosome (Chr), risk allele frequency (RAF).