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Mendelian Randomization and mediation analysis of leukocyte telomere length and risk of lung and head and neck cancers

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ABSTRACT

Background: Evidence from observational studies of telomere length (TL) has been conflicting regarding its direction of association with cancer risk. We investigated the causal relevance of TL for lung and head and neck cancers using Mendelian Randomization (MR) and mediation analyses.

Methods: We developed a novel genetic instrument for TL in chromosome 5p15.33, using variants identified through deep-sequencing, that were genotyped in 2051 cancer-free subjects. Next, we conducted an MR analysis of lung (16396 cases, 13013 controls) and head and neck cancer (4415 cases, 5013 controls) using 8 genetic instruments for TL. Lastly, the 5p15.33 instrument and distinct 5p15.33 lung cancer risk loci were evaluated using two-sample mediation analysis, to quantify their direct and indirect, telomere-mediated, effects.

Results: The multi-allelic 5p15.33 instrument explained 1.49-2.00% of TL variation in our data ($p=2.6\times10^{-9}$). The MR analysis estimated that a 1000 base pair increase in TL increases risk of lung cancer (OR=1.41, 95% CI: 1.20-1.65) and lung adenocarcinoma (OR=1.92, 95% CI: 1.51-2.22), but not squamous lung carcinoma (OR=1.04, 95% CI: 0.83-1.29), or head and neck cancers (OR=0.90, 95% CI: 0.70-1.05). Mediation analysis of the 5p15.33 instrument indicated an absence of direct effects on lung cancer risk (OR=1.00, 95% CI: 0.95-1.04). Analysis of distinct 5p15.33 susceptibility variants estimated that TL mediates up to 40% of the observed associations with lung cancer risk.

Conclusions: Our findings support a causal role for long telomeres in lung cancer etiology, particularly for adenocarcinoma, and demonstrate that telomere maintenance partially mediates the lung cancer susceptibility conferred by 5p15.33 loci.

KEY MESSAGES

- Genetic predisposition to long telomeres increases risk of lung cancer, predominately lung adenocarcinoma
- Genetic determinants of long telomeres are not associated with squamous carcinomas of the lung or head and neck
- Using two-sample mediation analysis we determined that the novel 5p15.33 instrument for telomere length does not have direct effects on the outcome, and demonstrated that the association between 5p15.33 lung cancer susceptibility variants is partially mediated by telomere length, suggesting the presence of other relevant mechanisms

INTRODUCTION

Telomeres are highly conserved stretches of tandem repeats of the TTAGGG sequence, which protect chromosome ends from degradation and maintain genome stability(1, 2). Due to the incomplete replication of chromosomes during cell division, human telomeres lose between 50 and 200 base pairs with each replication(1-3). In checkpoint proficient cells critically short telomeres trigger senescence, followed by apoptosis, which represents a barrier against cancer initiation by limiting cellular proliferation(4, 5). As telomeres shorten their ability to maintain chromosomal stability also diminishes, which may increase cancer susceptibility(6, 7). However, long telomeres may also promote cancer development through an accumulation of mutations due to prolonged cell survival and proliferation. In fact, cancer cells are characterized by such a proliferative advantage, often through reactivation of telomerase, which is normally silent in somatic cells(4, 5, 8).

Telomere length (TL) has been studied extensively in relation to cancer risk. However, findings of epidemiologic studies have been conflicting (6, 9-11). Observational studies investigating TL measured after cancer diagnosis are particularly vulnerable to reverse causation and residual confounding, therefore shorter TL observed in cancer cases is likely to reflect underlying disease or the impact of cancer treatment (12, 13). It is also difficult to isolate the influence of TL on cancer risk from that of other risk factors that influence both TL and cancer susceptibility, including biological or replicative age (10, 14, 15).

Mendelian Randomization (MR) is an approach for evaluating causality by using single nucleotide polymorphisms (SNPs) in relevant genes as instrumental variables (IVs) (16). Genome-wide association studies (GWAS) identified a number of genetic regions involved in TL regulation, including genes encoding the catalytic subunit of telomerase (TERT) in chromosome 5p15.33 and its RNA template (TERC) in 3q26.2 (17-21). By leveraging these associations, MR can provide a valid test of the causal hypothesis assuming the genetic IVs only affect cancer risk through TL regulation.

Previous studies using genetic proxies for TL suggest that longer telomeres confer an increased risk of lung cancer, especially adenocarcinoma (22-24), which is consistent with the

findings of prospective observational studies (25-27). Lung cancer case-control studies report both increased (28) and inverse (6, 29) associations for long TL, and some implicate high TL variability in lung cancer susceptibility (30). For head and neck cancers (HNC), which are predominantly squamous carcinomas, short TL is consistently associated with increased risk in case-control studies (6, 31, 32), whereas a recent MR analysis (24) did find evidence supporting a causal relationship.

The overarching aim of this study is to investigate the causal relationship between TL and risk of lung and upper aero-digestive tract cancers. First, we developed a novel genetic instrument for TL in chromosome 5p15.33, given the extensive pleiotropy in this region and potential for violating MR assumptions (22, 33). Next, we conducted the largest two-sample MR analysis of lung and HNC risk to date. Lastly, we quantified the direct and telomere-mediated effects of 5p15.33 genetic variants on cancer risk using a two-sample mediation analysis approach (Figure 1).

METHODS

Study populations

We used individual-level data from 23 pooled studies of lung cancer, with 16396 cases (5690 adenocarcinoma, 4045 squamous carcinoma) and 13013 controls; and 11 HNC studies with 4415 cases and 5013 controls, all part of the OncoArray collaboration (34) (Supplementary Tables 1-2). Descriptions of studies and genotyping methods have been previously published (34, 35) (details in Supplementary File 1). Analyses were restricted to individuals of predominantly European ancestry (≥80% lung, >70% HNC)(34, 36). Studies received approval from institutional research ethics review boards and informed consent was obtained from the participants.

The novel 5p15.33 instrument was developed using data from two studies: the cancer-free controls from the Mount Sinai and Princess Margaret Hospital (MSH-PMH) case-control study in Toronto(37), and cancer-free individuals from the Copenhagen General Population Study (CGPS)(38), a population-based prospective cohort (Table 1). TL was measured in DNA from peripheral blood leukocytes using previously described quantitative polymerase chain reaction assays performed in MSH-PMH (37) and CGPS (23, 38) (details in Supplementary File 2).

Statistical Analysis

Mendelian randomization analysis

The genetic instruments for TL included independent SNPs showing strong prior evidence of association with TL, such as $p<5\times10^{-8}$ in the discovery stage of at least one GWAS and replication in a separate GWAS or meta-analysis (17-21). In addition to the new 5p15.33 instrument described below, we selected 7 additional loci involved in telomere maintenance: rs10165485 (proxy for rs11125529, $r^2=1.0$) in ACYP2 (2p16.2), rs6772228 in PXK (3p14.3), rs10936599 in TERC (3q26.2), rs11100479 (proxy for rs7675998, $r^2=0.99$) in NAF1 (4q32.2), rs9420907 in OBFC1 (10q24.3), rs10419926 in ZNF676 (19p12), and rs755017 near RTEL1 and ZBTB46 (20q13). Only genotyped, non-imputed variants were used.

For the purpose of developing a new instrument in the 5p15.33 region, TL values were converted to Z-scores in MSH-PMH (n=879) and CGPS (n=1172) studies separately, and pooled to increase statistical power. Linear regression was used to estimate the association between 899 variants in 5p15.33 and TL, adjusting for age, sex, study, and the top 5 genetic ancestry principal components (PCs).

Selection of variants for the 5p15.33 instrument was based on statistical significance, consistency across the two studies, and instrument strength, measured by the F statistic, which depends on the variance in TL explained by the genetic predictors (R^2), sample size (n), and number of instruments (k): $F = \left(\frac{n-k-1}{k}\right)\left(\frac{R^2}{1-R^2}\right)$. Variants were considered for inclusion in the 5p15.33 instrument if they met the following criteria:

i. F≥5 and p<0.05 in the Toronto and Copenhagen combined dataset (n=2051)
ii. F<5 and p<0.05 overall (n=2051) and F>5 among never smokers (n=848)
iii. Consistent direction of allelic effects in MSH-PMH and CGPS
iv. Minor allele detected in at least 2 individuals

Independent genetic variants (r^2<0.2) that met the selection criteria were combined into an allele score representing the 5p15.33 region to increase the power of the resulting instrument (39, 40).

The MR analysis combined summary statistics across the genetic IVs to estimate the causal parameter $_{IV}$, which is the log odds ratio (OR) describing the causal effect of increasing TL on cancer risk (Supplementary Figure 1). Parameters for the MR analysis included $_{TL}$ and $_{Y}$, where $_{TL}$ is a vector of SNP-TL associations and $_{Y}$ is a vector of per-allele cancer log ORs for each instrument. For genetic instruments outside of 5p15.33, $_{TL}$ and corresponding standard errors (SE) were obtained from the literature and scaled to represent a 1000 base pair (kbp) increase in leukocyte TL, a proxy for TL in relevant tissues(19-21). For all instruments, $_{Y}$ and corresponding SE were estimated directly using individual-level OncoArray lung and HNC data. Logistic regression models were adjusted for age, sex, study, and 10 PCs.

The causal parameter $_{IV}$ was estimated using the maximum likelihood-based (ML) approach and the inverse-variance weighted (IVW) method (41, 42). This was complemented by sensitivity analyses using the weighted median estimator (WME), which provides valid estimates of the causal parameter even when up to 50% of the statistical weights are contributed by genetic instruments violate MR assumptions (43).

Mediation analysis

The aim of the mediation analysis was to quantify how much of the lung cancer association in the 5p15.33 region is mediated by TL. First, we validated the 5p15.33 instrument by decomposing its total effect on lung cancer into direct and indirect effects, mediated by TL. Next, we extended this analysis to independent (r^2<0.20) variants that capture the lung cancer association signal in 5p15.33 (details in Supplementary File 3).

Our mediation approach is based on the counterfactual framework(44, 45) and extends the sensitivity analysis using two randomized controlled trials proposed by Vanderweele, which allows

the mediator-outcome ($_2$) and exposure-mediator ($_1$) relationships to be estimated in separate studies (46). Application of this approach in the present context assumes that a valid estimate for the mediator-outcome relationship can be obtained from an independent MR or cohort studies. Based on previously published formulas for mediation analysis (44, 45), the total effect (TE) of increasing the exposure from reference level a^* to level a on lung cancer (Y) conditional on covariates c can be decomposed into natural direct effects (NDE) and natural indirect effects (NIE):

$$OR^{TE}_{a,a^*|c} = \frac{P(Y_a = 1|c)/\{1 - P(Y_a = 1|c)\}}{P(Y_{a^*} = 1|c)/\{1 - P(Y_{a^*} = 1|c)\}} = OR^{NIE}_{a,a^*|c} \times OR^{NDE}_{a,a^*|c} \quad (1)$$

Assuming a rare outcome and absence of exposure-mediator interaction, mediated effects are given by:

$$OR^{NIE}_{a,a^*|c} \approx \exp\{\theta_2 \times \beta_1(a - a^*)\} \quad (2)$$

where $_2$ is log-OR per one unit increment in TL and $_1$ is the effect of the 5p15.33 instrument on TL. Based on equation 1, NDE can be obtained by subtracting the NIE from the total effect:

$$\log(OR^{NDE}_{a,a^*|c}) \approx \log(OR^{TE}_{a,a^*|c}) - \log(OR^{NIE}_{a,a^*|c}) \quad (3)$$

In the presence of interaction between the exposure and mediator, the NIE is given by:

$$OR^{NIE}_{a,a^*|c} \approx \exp\{(\theta_2 \times \beta_1 + \theta_3 \times \beta_1 a) \times (a - a^*)\} \quad (4)$$

where $_2$ now represents the main effect of the mediator, TL, and $_3$ is the exposure-mediator interaction parameter, with NDE having a more complicated form given by Valeri and VanderWeele(45). Formulas for a dichotomized mediator are provided in Supplementary File 4.

The $_1$ parameter for the 5p15.33 instrument is equivalent to $_{TL}$ estimated in the cancer-free subset of the MSH-PMH and CGPS studies, adjusting for appropriate covariates. For 5p15.33 cancer susceptibility variants, $_1$ estimates were selected from Bojesen et al. (47), the largest fine-mapping analysis of common 5p15.33 loci and TL with 15567 cancer-free controls. Per allele associations were reported as percent increase in TL and base-pair change. OR^{TE} for all variants was estimated in 23 lung cancer OncoArray studies, and is equivalent to $_Y$ for the 5p15.33 instrument.

External estimates of the mediator-outcome relationship ($_2$) were substituted into the equation (2) to avoid estimating the effect of TL on lung cancer risk directly using MSH-PMH case-control data, which are likely to be biased due to the post-diagnostic timing of TL measurement. The effect of TL on lung cancer risk was obtained from two studies: an MR analysis TL by Zhang et al.(22), and a meta-analysis of prospective studies by Zhu et al. (11) (Supplementary Figure 2).

Since interaction between the 5p15.33 instrument and TL is plausible, we conducted sensitivity analyses under different magnitudes of $_3$ (details in Supplementary File 4). Confidence intervals for the NIE and NDE were approximated as Bayesian credible intervals. Analyses were conducted using R version 3.3.3.

RESULTS

Characteristics of the combined Toronto and Copenhagen dataset (n=2051), used to develop the 5p15.33 instrument, are summarized in Table 1. The cancer-free participants in the MSH-PMH and CGPS studies were of similar mean age, 61.0 and 61.30 years, respectively. Age was the strongest predictor of TL ($p=2.6\times10^{-30}$), while sex, smoking status, and cigarette pack-years among smokers were not associated with relative TL (Supplementary Table 3).

Novel 5p15.33 instrument for telomere length

The 5p15.33 variants comprising this instrument were not used in any previous MR studies of TL. After excluding 17 singletons and other SNPs that did not meet our criteria, 14 variants were included in the multi-allelic instrument for 5p15.33 (Table 2; regional plot and LD illustrated in Supplementary Figure 3). Most variants were located in non-coding intronic regions of several genes, including SLC6A3, TERT, LPCAT1, and a long-noncoding RNA (LINC01511) except for rs35033501, a synonymous TERT variant. The resulting multi-allelic 5p15.33 IV accounted for 1.49% of variation in the telomere Z-score in all subjects (F = 35.83; $_{TL}$ = 0.14, SE=0.02) and 2.00% in never smokers (F = 20.81), but was not predictive of smoking status (p=0.19) or cigarette pack-years among smokers (p=0.59) (Table 3). The 5p15.33 instrument was positively associated with lung cancer (OR=1.04,

95% CI: 1.01-1.07) and lung adenocarcinoma (OR=1.06, 1.03-1.10), but not squamous lung carcinomas (OR=1.03, 0.98-1.07). An inverse association was observed for HNC (OR=0.95, 0.90-1.00) and oral cavity cancer (OR=0.93, 0.87-0.98).

Telomere length and cancer risk

Results of the MR analysis based on 8 genetic instruments are presented in Table 4 and Figure 2. The likelihood-based model estimated a 41% increase in lung cancer risk per kbp increase in TL (OR_{ML}=1.41, 95% CI: 1.20-1.65). Estimates of the causal OR for lung cancer remained consistent across MR estimation methods. Genetic determinants of TL were predominantly associated with adenocarcinoma (OR_{ML}=1.92, 1.51-2.45), and appeared unrelated to squamous carcinoma (OR_{ML}=1.04, 0.83-1.29) and small cell carcinoma (OR_{ML}=1.03, 0.76-1.39).

The effect of long TL on lung cancer risk was larger in magnitude among never smokers (OR_{ML}=1.78, 1.22-2.61) compared to smokers (OR_{ML}=1.36, 1.14-1.63), although the former was attenuated in sensitivity analyses (OR_{WME}=1.55, 95% CI: 0.98-2.46). Effects on adenocarcinoma risk were also substantial in never smokers (OR_{ML}=2.68, 1.70-4.24). Genetic determinants of long telomeres conferred a 68% increase in lung cancer risk (OR_{ML}=1.68, 1.07-2.62) in subjects aged 50 years or younger. In contrast to lung cancer, genetic predisposition for longer TL did not seem related to risk of HNC overall (OR_{ML}= 0.90, 0.70-1.05), oral cavity (OR_{ML}=0.88, 0.65-1.19) and oropharynx cancers (OR_{ML}=0.83, 0.59-1.16).

Several additional sensitivity analyses were undertaken to further interrogate the MR results. Since smoking is an established risk factor for both HNC and lung cancer, MR analyses were repeated with adjustment for cigarette pack-years and smoking status. No appreciable changes were observed in the causal effect estimates for lung cancer overall (OR_{ML}=1.50, 1.27-1.78), lung adenocarcinoma (OR_{ML}=1.95, 1.53-2.49), HNC (OR_{ML}=0.91, 0.67-1.23), oral cavity (OR_{ML}=0.82, 0.57-1.18) or oropharynx cancers (OR_{ML}=0.86, 0.57-1.31).

The potential for directional pleiotropy was evaluated by checking for asymmetry in the plots depicting ratio estimates for each instrument, $_{Y}\big/_{TL}$, plotted against instrument strength,

$_{TL}/SE(\ _{Y})$ (Supplementary Figure 4). These results were not suggestive of pleiotropy and none of the genetic instruments were associated with cigarette smoking status or pack-years (Supplementary Table 4). Lastly, selected causal effects were re-estimated using the weighted mode-based estimator (MBE), which is robust to horizontal pleiotropy when the largest number of similar causal effect estimates are based on valid instruments, even if the majority of instruments are invalid (48). Estimates for lung cancer overall (OR_{MBE}=1.34, 1.08-1.66), lung adenocarcinoma (OR_{MBE}=1.55, 1.14-2.12), and adenocarcinoma in never smokers (OR_{MBE}=2.04, 1.04-4.04), were consistent with the primary results in Table 4.

Mediation analysis of the 5p15.33 instrument

We conducted mediation analyses to quantify direct (OR^{NDE}) and indirect effects (OR^{NIE}) of the 5p15.33 instrument on lung cancer. The OR^{NIE} we report is the proportional change in the odds of lung cancer for a change in TL that occurs when the 5p15.33 allele score increases by one from the reference level, corresponding to the mean of the allele score distribution. The estimate of the TL effect on lung cancer ($_{2}$) was selected from the strict model reported by Zhang et al.(22) (OR per kbp increase: 1.37, 95% CI: 1.12-1.68), which excluded rs2736100 (TERT). OR^{TE} for the 5p15.33 IV was re-estimated after removing overlapping subjects (n=3498) between the OncoArray and Zhang et al.(22). Assuming no interaction between the 5p15.33 IV and TL, the lung cancer effect appeared to be almost entirely mediated by TL (OR^{NIE}=1.05, 1.01-1.08), whereas the direct effects of the 5p15.33 IV appeared null (OR^{NDE}=1.00, 0.95-1.04) (Figure 3; Supplementary Table 5). For lung adenocarcinoma, the 5p15.33 effects mediated by TL were larger in magnitude (OR^{NIE}=1.11, 1.05-1.18) than direct effects, which were close to unity (OR^{NDE}=0.97, 0.90-1.03).

Interaction sensitivity analyses for the NIE and NDE were carried out across three levels of $_{3}$: 0.10, 0.20 and 0.30. As the magnitude of the interaction parameter increased, so did the NIE, while TL-independent effects were not observed (Figure 3). Indirect effects on lung cancer risk mediated by TL ranged from OR^{NIE}=1.06 (95% CI: 1.03-1.10) for $_{3}$=0.10, to OR^{NIE}=1.09 (95% CI:

1.05-1.15) for $_3$= 0.30. For adenocarcinoma, increasing the magnitude of interaction between the 5p15.33 IV and TL was also associated with increasing NIE and diminishing direct effects.

The prospective meta-analysis estimate of $_2$ from Zhu et al.(11) reported an OR of 1.28 (95% CI: 1.09-1.50) for lung cancer comparing long vs. short TL. Based on this binary mediator, the NIE mediated by TL was attenuated, but remained statistically significant (OR^{NIE}=1.01, 1.00-1.03). A positive direct effect on lung cancer risk was also observed (OR^{NDE}=1.03, 1.00-1.06). Assuming interaction between the 5p15.33 instrument and TL, the mediated effects ranged from OR^{NIE}=1.02 (95% CI: 1.01-1.03) when $_3$=0.10, to OR^{NIE}=1.03 (95% CI: 1.01-1.05) when $_3$=0.30, while the direct effects decreased (Figure 3; Supplementary Table 5).

Mediation analysis of 5p15.33 lung cancer susceptibility loci

Five common (MAF>0.05), independent (r^2 <0.20) variants were selected to represent the lung cancer susceptibility signal in 5p15.33 (details in Supplementary File 3): rs7705526 ($P_{Adeno}=4.6\times10^{-13}$; $P_{Lung}=8.0\times10^{-7}$), rs2736108 ($P_{Adeno}=1.7\times10^{-12}$; $P_{Lung}=1.8\times10^{-11}$), rs421629 ($P_{Adeno}=6.2\times10^{-9}$; $P_{Lung}=1.2\times10^{-16}$), rs13167280 ($P_{Adeno}=1.4\times10^{-8}$; $P_{Lung}=1.1\times10^{-6}$), and rs56345976 ($P_{Adeno}=2.2\times10^{-7}$; $P_{Lung}=3.6\times10^{-9}$). These variants have been associated with lung cancer and lung adenocarcinoma in previous studies (37, 49-51), and are representative of the genetic susceptibility architecture in this region.

Estimates of $_1$ were obtained from Bojesen et al.(47), and three TERT lung cancer risk variants were significantly associated with TL: rs7705526 ($P_{TL}=2.3\times10^{-14}$), rs2736108 ($P_{TL}=5.8\times10^{-7}$), and rs13167280 ($P_{TL}=1.2\times10^{-5}$). Estimates of $_2$ were selected from the MR analysis (22) and OR^{TE} were re-estimated for each variant after removing the overlapping subjects. For all variants, the TL-increasing allele was positively associated with cancer risk, and both direct and indirect, TL-mediated effects were significant (Supplementary Table 6).

For lung cancer, the proportion mediated (PM) by TL was the largest for rs13167280 (OR^{NIE}=1.05, 1.03-1.07; PM=40.5%), followed by rs7705526 (OR^{NIE}=1.03, 1.01-1.05; PM=28.7%)

and rs2736108 (OR^{NIE} 1.02, 1.01-1.03; PM=13.7%). The magnitude and proportion of the SNP effects that were mediated by TL were larger for adenocarcinoma compared to lung cancer overall: rs7705526 (OR^{NIE}=1.07, 1.04-1.10; PM=36.5%), rs13167280 (OR^{NIE}=1.05, 1.03-1.07; PM=24.8%), and rs2736108 (OR^{NIE}=1.04, 1.03-1.06; PM=22.9%).

DISCUSSION

We observed an association between genetic determinants of long telomeres and increased risk of lung, but not head and neck cancers. Our findings lend support to a causal relationship between longer leukocyte TL and increased risk of lung adenocarcinoma, but not squamous or small cell carcinoma. The magnitude of the increased risk was larger in never smokers and participants aged 50 or younger, consistent with a stronger influence of genetic susceptibility in individuals with a lower burden of modifiable risk factors (52). Although histology and smoking status are closely linked, our results suggest that the associations were histology-specific for adenocarcinoma (53, 54). Lastly, our mediation analysis demonstrated that mechanisms resulting in long telomeres mediate a proportion of the increase in lung cancer and lung adenocarcinoma risk conferred by 5p15.33 loci, and that the proportion of genetic susceptibility attributed to telomere maintenance differs between distinct 5p15.33 susceptibility loci.

Other analyses using multi-SNP telomere scores have also observed excess risks of lung cancer(22-24) and lung adenocarcinoma(22, 24), but did not observe an effect of TL on oral cancer risk (23, 24). Opposite directions of effect for the 5p15.33 instrument on lung and HNC are consistent with earlier reports of opposing allelic effects for 5p15.33 SNPs on lung and oral cancer, respectively (35, 55). Leukocyte TL and functional TERT variants were previously reported to be unrelated to squamous HNC risk(56), although one study linked short TL to increased HNC risk based on rs2736100, which may be an invalid instrument(22, 57). With the exception of the 5p15.33 IV, the instruments used in this study overlap with those used in other MR analyses of TL (22-24).

Our findings lend support to the hypothesis that a greater number of telomere-increasing alleles increase lung cancer susceptibility. Although the precise molecular mechanisms remain to be elucidated, telomere maintenance may promote carcinogenesis by enabling prolonged cell survival

and accumulation of mutations. This is supported by the hallmark observation that telomerase is overexpressed in 85-90% of adult tumors(8, 58), as well as recent data showing that long telomeres increase chromosomal instability(59) and promote immortalization of cancer cells(60). Excessively long telomeres may also be more fragile and dysfunctional, which is supported by the observation that TERT not only replenishes telomeres, but also regulates a trimming process to maintain TL homeostasis (61-63).

Differences in the effect of TL persisted after stratifying by smoking status, suggesting that underlying mechanisms differ across tissues and histological types. Longer TL does not appear to increase risk of small cell lung cancer or squamous lung carcinoma, the histology that also comprises 90% of HNC tumours, and for which the causal effect of tobacco smoking is the strongest(64). Since our genetic instruments are unrelated to smoking, confounding is unlikely to account for these differences. It is plausible that genetic predisposition for telomere maintenance offers some protection against genomic instability due to oxidative stress, declining regenerative capacity and immune function(7, 65, 66). Although human papillomavirus (HPV), a known cause of oropharynx cancer(67), has been reported to correlate with TL(31), the similarity of associations observed for oropharynx and oral cancers, only 2% of which are attributed to HPV(68), suggests that HPV infection is unlikely to modify the influence of TL.

This analysis has several important strengths. Genetic instruments represent are unaffected by reverse causality and are more likely to reflect causality due to the independence of genotypes from confounding factors. In addition to the large sample size, our analysis leveraged rich genetic data in 5p15.33, including rare sequence variations, to develop a robust, novel instrument. Furthermore, the use of multiple genetic instruments from essential genes for telomere maintenance mitigates the possibility for weak instruments bias and genetic confounding due to pleiotropy. The association between genetic predisposition to long TL and increased lung cancer risk persisted in analyses using the weighted median and mode-based estimators, which further supports the causal interpretation of these results.

Our mediation analysis offers insight not only by validating the new 5p15.33 instrument, by demonstrating an absence of direct effects, but also by formally quantifying the contribution of telomere-related mechanisms to the observed association between the established lung and adenocarcinoma susceptibility loci and lung cancer risk in this region. Although we confirmed that TL is an important molecular mechanism underlying the associations observed for 5p15.33 lung cancer risk loci, our results also indicated that only a fraction of these genetic effects operate through telomere maintenance. For instance, only 3-8% of the total effect of rs421629 (CLPTM1L) was mediated TL, and approximately half of the association between the TERT loci and lung cancer risk can be attributed to telomere mechanisms.

These findings are consistent with our knowledge that 5p15.33 is a complex susceptibility locus for multiple cancers(33, 55, 69) and GWAS peaks in this region also encompass non-cancer traits, such as red blood cell counts, prostate-specific antigen levels, and lung diseases(69-72). In addition, non-canonical functions of TERT, related to proliferation and differentiation via regulation of Wnt/β-catenin and Myc signaling, have been proposed(73). Therefore, although telomere maintenance is clearly an important 5p15.33 mechanism, cancer susceptibility loci in this region likely invoke additional pathways.

Several limitations of this work should be acknowledged. The time lag between genotype assignment at conception and the assessment of genetic effects on TL and cancer risk, as well as the time-varying nature of TL, pose challenges for interpreting MR estimates of the causal effect (74). However, while genetic instruments do not recapitulate all aspects of telomere function and dynamics, they can still provide a valid test of the causal hypothesis that inherited predisposition to telomere maintenance increases lung cancer susceptibility (75). Secondly, genetic instruments for leukocyte TL may not be accurate proxies for TL in target tissues, which would reduce the power of our genetic instruments. However, the validity of instruments based on leukocyte TL is supported by correlation between TL in leukocytes and other tissues, including lung, and comparable rates of telomere shortening across somatic tissues (76-78). Thirdly, our MR analysis may be affected by winner's curse, with the magnitude and strength of association with TL observed in the discovery dataset likely

to be exaggerated, particularly the 5p15.33 instrument. However, since the instrument discovery and MR analysis populations are independent, any potential bias in the causal parameter due to winner's curse or limited instrument strength will be towards the null (79). A related concern involves our ability to detect subtle effects of TL on cancer risk due to the modest proportion of variation in TL explained by our genetic instruments (approximately 5%), which is comparable to most genetic instruments for complex phenotypes (80-82). Based on our power calculations, this analysis was adequately powered (>80%) to detect effects with OR of 1.5 and above for all lung and HNC histological subtypes and smoking-stratified analyses.

Lastly, the validity of our mediation analysis depends in part on the validity of the published estimates of the mediator-outcome relationship. MR-based estimates of the mediator-outcome relationship are likely to satisfy the assumption of no unmeasured confounding, but must assume that all instruments used in Zhang et al. (22) were valid. While observational studies are more susceptible to confounding and bias due measurement error in the molecular mediator (83), a synthesis of prospective studies provides complementary evidence that does not depend on MR assumptions, and is less vulnerable to reverse causation than case-control designs.

In summary, we demonstrated that genetic determinants of long telomeres are associated with an increased risk of lung cancer, particularly adenocarcinoma. The associations observed for HNC were less consistent with a causal relationship, however we cannot preclude the possibility of a very subtle telomere effects (OR<1.5). Using mediation analysis that incorporates independent published data, we validated the novel 5p15.33 instrument and quantified the proportion of the lung cancer association signal in 5p15.33 that is mediated by TL. While this work provides insight into the role of TL in cancer etiology, further research is needed to identify appropriate ways of utilizing this complex biomarker in the context of disease prevention or clinical intervention.

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References

1. Blackburn EH. Structure and function of telomeres. Nature. 1991;350(6319):569-73.

2. de Lange T. Protection of mammalian telomeres. Oncogene. 2002;21(4):532-40.

3. Zhao Y, Sfeir AJ, Zou Y, Buseman CM, Chow TT, Shay JW, et al. Telomere extension occurs at most chromosome ends and is uncoupled from fill-in in human cancer cells. Cell. 2009;138(3):463-75.

4. Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, et al. Extension of life-span by introduction of telomerase into normal human cells. Science. 1998;279(5349):349-52.

5. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646-74.

6. Wu X, Amos CI, Zhu Y, Zhao H, Grossman BH, Shay JW, et al. Telomere dysfunction: a potential cancer predisposition factor. J Natl Cancer Inst. 2003;95(16):1211-8.

7. Bernardes de Jesus B, Blasco MA. Telomerase at the intersection of cancer and aging. Trends in genetics : TIG. 2013;29(9):513-20.

8. Newbold RF. The significance of telomerase activation and cellular immortalization in human cancer. Mutagenesis. 2002;17(6):539-50.

9. Wentzensen IM, Mirabello L, Pfeiffer RM, Savage SA. The association of telomere length and cancer: a meta-analysis. Cancer Epidemiol Biomarkers Prev. 2011;20(6):1238-50.

10. Prescott J, Wentzensen IM, Savage SA, De Vivo I. Epidemiologic evidence for a role of telomere dysfunction in cancer etiology. Mutat Res. 2012;730(1-2):75-84.

11. Zhu X, Han W, Xue W, Zou Y, Xie C, Du J, et al. The association between telomere length and cancer risk in population studies. Scientific reports. 2016;6:22243.

12. Benitez-Buelga C, Sanchez-Barroso L, Gallardo M, Apellaniz-Ruiz M, Inglada-Perez L, Yanowski K, et al. Impact of chemotherapy on telomere length in sporadic and familial breast cancer patients. Breast Cancer Res Treat. 2015;149(2):385-94.

13. Li P, Hou M, Lou F, Bjorkholm M, Xu D. Telomere dysfunction induced by chemotherapeutic agents and radiation in normal human cells. Int J Biochem Cell Biol. 2012;44(9):1531-40.

14. Huzen J, Wong LS, van Veldhuisen DJ, Samani NJ, Zwinderman AH, Codd V, et al. Telomere length loss due to smoking and metabolic traits. J Intern Med. 2014;275(2):155-63.

15. Bojesen SE. Telomeres and human health. J Intern Med. 2013;274(5):399-413.

16. Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. Human molecular genetics. 2014;23(R1):R89-98.

17. Levy D, Neuhausen SL, Hunt SC, Kimura M, Hwang SJ, Chen W, et al. Genome-wide association identifies OBFC1 as a locus involved in human leukocyte telomere biology. Proc Natl Acad Sci U S A. 2010;107(20):9293-8.

18. Prescott J, Kraft P, Chasman DI, Savage SA, Mirabello L, Berndt SI, et al. Genome-wide association study of relative telomere length. PLoS One. 2011;6(5):e19635.

19. Mangino M, Hwang SJ, Spector TD, Hunt SC, Kimura M, Fitzpatrick AL, et al. Genome-wide meta-analysis points to CTC1 and ZNF676 as genes regulating telomere homeostasis in humans. Human molecular genetics. 2012;21(24):5385-94.

20. Pooley KA, Bojesen SE, Weischer M, Nielsen SF, Thompson D, Amin Al Olama A, et al. A genome-wide association scan (GWAS) for mean telomere length within the COGS project: identified loci show little association with hormone-related cancer risk. Human molecular genetics. 2013;22(24):5056-64.

21. Codd V, Nelson CP, Albrecht E, Mangino M, Deelen J, Buxton JL, et al. Identification of seven loci affecting mean telomere length and their association with disease. Nat Genet. 2013;45(4):422-7, 7e1-2.

22. Zhang C, Doherty JA, Burgess S, Hung RJ, Lindstrom S, Kraft P, et al. Genetic determinants of telomere length and risk of common cancers: a Mendelian randomization study. Human molecular genetics. 2015;24(18):5356-66.

23. Rode L, Nordestgaard BG, Bojesen SE. Long telomeres and cancer risk among 95 568 individuals from the general population. International journal of epidemiology. 2016;45(5):1634-43.

24. The Telomeres Mendelian Randomization Collaboration. Association Between Telomere Length and Risk of Cancer and Non-Neoplastic Diseases: A Mendelian Randomization Study. JAMA Oncology. 2017;3(5):636-51.

25. Seow WJ, Cawthon RM, Purdue MP, Hu W, Gao YT, Huang WY, et al. Telomere length in white blood cell DNA and lung cancer: a pooled analysis of three prospective cohorts. Cancer research. 2014;74(15):4090-8.

26. Lan Q, Cawthon R, Gao Y, Hu W, Hosgood HD, 3rd, Barone-Adesi F, et al. Longer telomere length in peripheral white blood cells is associated with risk of lung cancer and the rs2736100 (CLPTM1L-TERT) polymorphism in a prospective cohort study among women in China. PLoS One. 2013;8(3):e59230.

27. Shen M, Cawthon R, Rothman N, Weinstein SJ, Virtamo J, Hosgood HD, 3rd, et al. A prospective study of telomere length measured by monochrome multiplex quantitative PCR and risk of lung cancer. Lung cancer. 2011;73(2):133-7.

28. Sanchez-Espiridion B, Chen M, Chang JY, Lu C, Chang DW, Roth JA, et al. Telomere length in peripheral blood leukocytes and lung cancer risk: a large case-control study in Caucasians. Cancer research. 2014;74(9):2476-86.

29. Jang JS, Choi YY, Lee WK, Choi JE, Cha SI, Kim YJ, et al. Telomere length and the risk of lung cancer. Cancer science. 2008;99(7):1385-9.

30. Sun B, Wang Y, Kota K, Shi Y, Motlak S, Makambi K, et al. Telomere length variation: A potential new telomere biomarker for lung cancer risk. Lung cancer. 2015;88(3):297-303.

31. Zhang Y, Sturgis EM, Dahlstrom KR, Wen J, Liu H, Wei Q, et al. Telomere length in peripheral blood lymphocytes contributes to the development of HPV-associated oropharyngeal carcinoma. Cancer research. 2013;73(19):5996-6003.

32. Bau DT, Lippman SM, Xu E, Gong Y, Lee JJ, Wu X, et al. Short telomere lengths in peripheral blood leukocytes are associated with an increased risk of oral premalignant lesion and oral squamous cell carcinoma. Cancer. 2013;119(24):4277-83.

33. Wang Z, Zhu B, Zhang M, Parikh H, Jia J, Chung CC, et al. Imputation and subset-based association analysis across different cancer types identifies multiple independent risk loci in the TERT-CLPTM1L region on chromosome 5p15.33. Human molecular genetics. 2014.

34. Amos CI, Dennis J, Wang Z, Byun J, Schumacher FR, Gayther SA, et al. The OncoArray Consortium: a Network for Understanding the Genetic Architecture of Common Cancers. Cancer Epidemiol Biomarkers Prev. 2016.

35. Lesseur C, Diergaarde B, Olshan AF, Wunsch-Filho V, Ness AR, Liu G, et al. Genome-wide association analyses identify new susceptibility loci for oral cavity and pharyngeal cancer. Nat Genet. 2016;48(12):1544-50.

36. Li Y, Byun J, Cai G, Xiao X, Han Y, Cornelis O, et al. FastPop: a rapid principal component derived method to infer intercontinental ancestry using genetic data. BMC bioinformatics. 2016;17:122.

37. Kachuri L, Amos CI, McKay JD, Johansson M, Vineis P, Bueno-de-Mesquita HB, et al. Fine mapping of chromosome 5p15.33 based on a targeted deep sequencing and high density genotyping identifies novel lung cancer susceptibility loci. Carcinogenesis. 2016;37(1):96-105.

38. Weischer M, Bojesen SE, Cawthon RM, Freiberg JJ, Tybjaerg-Hansen A, Nordestgaard BG. Short telomere length, myocardial infarction, ischemic heart disease, and early death. Arterioscler Thromb Vasc Biol. 2012;32(3):822-9.

39. Burgess S, Thompson SG. Use of allele scores as instrumental variables for Mendelian randomization. International journal of epidemiology. 2013;42(4):1134-44.

40. Pierce BL, Ahsan H, Vanderweele TJ. Power and instrument strength requirements for Mendelian randomization studies using multiple genetic variants. International journal of epidemiology. 2011;40(3):740-52.

41. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. Genetic epidemiology. 2013;37(7):658-65.

42. Thompson JR, Minelli C, Abrams KR, Tobin MD, Riley RD. Meta-analysis of genetic studies using Mendelian randomization--a multivariate approach. Statistics in medicine. 2005;24(14):2241-54.

43. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. Genetic epidemiology. 2016;40(4):304-14.

44. VanderWeele TJ. A three-way decomposition of a total effect into direct, indirect, and interactive effects. Epidemiology. 2013;24(2):224-32.

45. Valeri L, Vanderweele TJ. Mediation analysis allowing for exposure-mediator interactions and causal interpretation: theoretical assumptions and implementation with SAS and SPSS macros. Psychol Methods. 2013;18(2):137-50.

46. VanderWeele TJ. Explanation in Causal Inference: Methods for Mediation and Interaction. New York, NY: Oxford University Press; 2015.

47. Bojesen SE, Pooley KA, Johnatty SE, Beesley J, Michailidou K, Tyrer JP, et al. Multiple independent variants at the TERT locus are associated with telomere length and risks of breast and ovarian cancer. Nat Genet. 2013;45(4):371-84, 84e1-2.

48. Hartwig FP, Davey Smith G, Bowden J. Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption. International journal of epidemiology. 2017;46(6):1985-98.

49. McKay JD, Hung RJ, Gaborieau V, Boffetta P, Chabrier A, Byrnes G, et al. Lung cancer susceptibility locus at 5p15.33. Nat Genet. 2008;40(12):1404-6.

50. Pande M, Spitz MR, Wu X, Gorlov IP, Chen WV, Amos CI. Novel genetic variants in the chromosome 5p15.33 region associate with lung cancer risk. Carcinogenesis. 2011;32(10):1493-9.

51. McKay JD, Hung RJ, Han Y, Zong X, Carreras-Torres R, Christiani DC, et al. Large-scale association analysis identifies new lung cancer susceptibility loci and heterogeneity in genetic susceptibility across histological subtypes. Nat Genet. 2017;49(7):1126-32.

52. Brennan P, Hainaut P, Boffetta P. Genetics of lung-cancer susceptibility. The lancet oncology. 2011;12(4):399-408.

53. Samet JM, Avila-Tang E, Boffetta P, Hannan LM, Olivo-Marston S, Thun MJ, et al. Lung cancer in never smokers: clinical epidemiology and environmental risk factors. Clinical cancer research : an official journal of the American Association for Cancer Research. 2009;15(18):5626-45.

54. Couraud S, Zalcman G, Milleron B, Morin F, Souquet PJ. Lung cancer in never smokers--a review. European journal of cancer. 2012;48(9):1299-311.

55. Rafnar T, Sulem P, Stacey SN, Geller F, Gudmundsson J, Sigurdsson A, et al. Sequence variants at the TERT-CLPTM1L locus associate with many cancer types. Nat Genet. 2009;41(2):221-7.

56. Liu Z, Ma H, Wei S, Li G, Sturgis EM, Wei Q. Telomere length and TERT functional polymorphisms are not associated with risk of squamous cell carcinoma of the head and neck. Cancer Epidemiol Biomarkers Prev. 2011;20(12):2642-5.

57. Gu Y, Yu C, Miao L, Wang L, Xu C, Xue W, et al. Telomere length, genetic variants and risk of squamous cell carcinoma of the head and neck in Southeast Chinese. Scientific reports. 2016;6:20675.

58. Shay JW, Bacchetti S. A survey of telomerase activity in human cancer. European journal of cancer. 1997;33(5):787-91.

59. Bull CF, Mayrhofer G, O'Callaghan NJ, Au AY, Pickett HA, Low GK, et al. Folate deficiency induces dysfunctional long and short telomeres; both states are associated with hypomethylation and DNA damage in human WIL2-NS cells. Cancer prevention research. 2014;7(1):128-38.

60. Borah S, Xi L, Zaug AJ, Powell NM, Dancik GM, Cohen SB, et al. Cancer. TERT promoter mutations and telomerase reactivation in urothelial cancer. Science. 2015;347(6225):1006-10.

61. Zheng YL, Zhang F, Sun B, Du J, Sun C, Yuan J, et al. Telomerase enzymatic component hTERT shortens long telomeres in human cells. Cell Cycle. 2014;13(11):1765-76.

62. Martinez P, Thanasoula M, Munoz P, Liao C, Tejera A, McNees C, et al. Increased telomere fragility and fusions resulting from TRF1 deficiency lead to degenerative pathologies and increased cancer in mice. Genes & development. 2009;23(17):2060-75.

63. Rivera T, Haggblom C, Cosconati S, Karlseder J. A balance between elongation and trimming regulates telomere stability in stem cells. Nat Struct Mol Biol. 2017;24(1):30-9.

64. Pai SI, Westra WH. Molecular pathology of head and neck cancer: implications for diagnosis, prognosis, and treatment. Annu Rev Pathol. 2009;4:49-70.

65. von Zglinicki T. Role of oxidative stress in telomere length regulation and replicative senescence. Ann N Y Acad Sci. 2000;908:99-110.

66. Hohensinner PJ, Goronzy JJ, Weyand CM. Telomere dysfunction, autoimmunity and aging. Aging Dis. 2011;2(6):524-37.

67. Gillison ML, Chaturvedi AK, Anderson WF, Fakhry C. Epidemiology of Human Papillomavirus-Positive Head and Neck Squamous Cell Carcinoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2015;33(29):3235-42.

68. de Martel C, Plummer M, Vignat J, Franceschi S. Worldwide burden of cancer attributable to HPV by site, country and HPV type. International journal of cancer Journal international du cancer. 2017.

69. Wu YH, Graff RE, Passarelli MN, Hoffman JD, Ziv E, Hoffmann TJ, et al. Identification of Pleiotropic Cancer Susceptibility Variants from Genome-Wide Association Studies Reveals Functional Characteristics. Cancer Epidemiol Biomarkers Prev. 2018;27(1):75-85.

70. Gudmundsson J, Besenbacher S, Sulem P, Gudbjartsson DF, Olafsson I, Arinbjarnarson S, et al. Genetic correction of PSA values using sequence variants associated with PSA levels. Sci Transl Med. 2010;2(62):62ra92.

71. Kamatani Y, Matsuda K, Okada Y, Kubo M, Hosono N, Daigo Y, et al. Genome-wide association study of hematological and biochemical traits in a Japanese population. Nat Genet. 2010;42(3):210-5.

72. Fingerlin TE, Murphy E, Zhang W, Peljto AL, Brown KK, Steele MP, et al. Genome-wide association study identifies multiple susceptibility loci for pulmonary fibrosis. Nat Genet. 2013;45(6):613-20.

73. Low KC, Tergaonkar V. Telomerase: central regulator of all of the hallmarks of cancer. Trends Biochem Sci. 2013;38(9):426-34.

74. Swanson SA, Tiemeier H, Ikram MA, Hernan MA. Nature as a Trialist?: Deconstructing the Analogy Between Mendelian Randomization and Randomized Trials. Epidemiology. 2017;28(5):653-9.

75. VanderWeele TJ, Tchetgen Tchetgen EJ, Cornelis M, Kraft P. Methodological challenges in mendelian randomization. Epidemiology. 2014;25(3):427-35.

76. Friedrich U, Griese E, Schwab M, Fritz P, Thon K, Klotz U. Telomere length in different tissues of elderly patients. Mech Ageing Dev. 2000;119(3):89-99.

77. Saferali A, Lee J, Sin DD, Rouhani FN, Brantly ML, Sandford AJ. Longer telomere length in COPD patients with alpha1-antitrypsin deficiency independent of lung function. PLoS One. 2014;9(4):e95600.

78. Daniali L, Benetos A, Susser E, Kark JD, Labat C, Kimura M, et al. Telomeres shorten at equivalent rates in somatic tissues of adults. Nat Commun. 2013;4:1597.

79. Haycock PC, Burgess S, Wade KH, Bowden J, Relton C, Davey Smith G. Best (but oft-forgotten) practices: the design, analysis, and interpretation of Mendelian randomization studies. The American journal of clinical nutrition. 2016;103(4):965-78.

80. Hartwig FP, Borges MC, Horta BL, Bowden J, Davey Smith G. Inflammatory Biomarkers and Risk of Schizophrenia: A 2-Sample Mendelian Randomization Study. JAMA psychiatry. 2017;74(12):1226-33.

81. Carreras-Torres R, Johansson M, Haycock PC, Wade KH, Relton CL, Martin RM, et al. Obesity, metabolic factors and risk of different histological types of lung cancer: A Mendelian randomization study. PLoS One. 2017;12(6):e0177875.

82. Dimitrakopoulou VI, Tsilidis KK, Haycock PC, Dimou NL, Al-Dabhani K, Martin RM, et al. Circulating vitamin D concentration and risk of seven cancers: Mendelian randomisation study. Bmj. 2017;359:j4761.

83. Richmond RC, Hemani G, Tilling K, Davey Smith G, Relton CL. Challenges and novel approaches for investigating molecular mediation. Human molecular genetics. 2016;25(R2):R149-R56.