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Genetic variants related to longer telomere length are associated with increased risk of renal cell carcinoma

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Abstract

Background: Relative telomere length in peripheral blood leukocytes has been evaluated as a potential biomarker for renal cell carcinoma (RCC) risk in several studies, with conflicting findings.

Objective: We performed an analysis of genetic variants associated with leukocyte telomere length to assess the relationship between telomere length and RCC risk using Mendelian randomization, an approach unaffected by biases from temporal variability and reverse causation that might have affected earlier investigations.

Design, Setting, and Participants: Genotypes from nine telomere length associated variants for 10,784 cases and 20,406 cancer-free controls from six genome-wide association studies (GWAS) of RCC were aggregated into a weighted genetic risk score (GRS) predictive of leukocyte telomere length.

Outcome Measurements and Statistical Analysis: Odds ratios (ORs) relating the GRS and RCC risk were computed in individual GWAS datasets and combined by meta-analysis.

Results and Limitations: Longer genetically inferred telomere length was associated with an increased risk of RCC (OR=2.07 per predicted kilobase increase, 95% CI=1.70-2.53; P<0.0001). As a sensitivity analysis, we excluded two telomere length variants in linkage disequilibrium (R^2>0.5) with GWAS-identified RCC risk variants (rs10936599 and rs9420907) from the telomere length GRS; despite this exclusion, a statistically significant association between the GRS and RCC risk persisted (OR=1.73, 95% CI=1.36-2.21, P<0.0001). Exploratory analyses for individual histologic subtypes suggested comparable associations with the telomere length GRS for clear cell (N=5,573; OR=1.93, 95% CI=1.50-2.49, P<0.0001), papillary (N=573; OR=1.96,

95% CI=1.01-3.81, P=0.046) and chromophobe RCC (N=203; OR=2.37, 95% CI=0.78-7.17, P=0.13).

Conclusions: Our investigation adds to the growing body of evidence indicating some aspect of longer telomere length is important for RCC risk.

Patient Summary: Telomeres are segments of DNA at chromosome ends that maintain chromosomal stability. Our study investigated the relationship between genetic variants associated with telomere length and RCC risk. We found evidence suggesting individuals with inherited predisposition to longer telomere length are at increased risk of developing RCC.

Introduction

Telomeres are TTAGGG nucleotide repeats and a protein complex at chromosome ends that play an essential role in maintaining chromosomal stability. Due to the inability of DNA polymerase to fully extend 3' DNA ends, telomeres become gradually shorter with each cell division in the absence of telomerase activity[1]. Although in normal cells critically short telomeres will trigger cellular senescence and death, cancer cells can continue to divide despite telomere shortening and the resultant genomic instability[2]. Alternatively, upregulated telomerase activity leading to increased telomere length may also promote tumorigenesis by conferring properties of immortal growth[3]. Indeed, recent studies suggest longer telomere length may be a risk factor for select tumor types including melanoma, lung cancer, chronic lymphocytic leukemia, glioma and ovarian cancer[4-7].

As such, relative telomere length in peripheral blood leukocytes has been evaluated in numerous population-based studies as a suspected marker of cancer risk[8]. Most of these studies have characterized telomere length using multiplex quantitative polymerase chain reaction (qPCR) assays[9]. Results of studies of leukocyte telomere length and risk of renal cell carcinoma (RCC) have been inconsistent. Two small hospital-based case-control studies reported inverse associations between telomere length and risk of RCC[10, 11], whereas no significant evidence of an association was observed in a larger population-based case-control study[12] and two cohort-based investigations using pre-diagnostic samples[13, 14]. In contrast, longer leukocyte telomere length has been associated with reduced RCC survival[15]. Telomerase activity is elevated in renal tumors compared to adjacent normal renal tissue and has been associated with clinicopathologic features of advanced disease[16, 17].

These previous studies have several limitations. Leukocyte telomere length measurements in case-control studies, using post-diagnosis blood samples, may have been influenced by effects of the disease. All studies measured telomere length from a single time point, which may not adequately reflect telomere length status in the etiologically relevant time window, and were susceptible to confounding from RCC risk factors that may be associated with telomere length such as smoking[13, 18] and obesity[19]. Furthermore, qPCR-based measurements of telomere length are sensitive to pre-analytic factors such as DNA source material and extraction method[12, 20, 21].

Nine common genetic variants have been identified in genome-wide association studies (GWAS) that are associated with leukocyte telomere length at a level of genome-wide significance ($P<5\times10^{-8}$)[22-24]. Recent studies have evaluated the relationship between these genetic proxies of telomere length and risk of cancer and found evidence suggesting longer genetically inferred telomere length is associated with increased cancer risk[4-7]. The approach employed by these studies, Mendelian randomization, uses genetic variants associated with leukocyte telomere length as genetic instruments to investigate the relationship between leukocyte telomere length and RCC risk. For resulting effect estimates to have a valid causal interpretation, several conditions must hold: (1) the telomere length associated variants must be associated with telomere length in circulating leukocytes, (2) the telomere length associated variants should not be associated with other factors that are associated with telomere length and RCC risk and (3) the telomere length associated variants can only influence RCC risk by their effect on telomere length, that is they cannot have pleiotropic effects. An advantage of this approach is that it is not susceptible to the biases associated with measured telomere length as described above. A recent investigation surveying several chronic conditions suggested a

marginal positive association (P=0.01) between genetically predicted telomere length and RCC risk, although the sample size was smaller (N=2,461 RCC cases)[7].

In the present study, we evaluated RCC risk in relation to individual telomere length-related genetic variants and an aggregate genetic risk score (GRS) of telomere length associated genetic variants in a large sample of six RCC GWAS datasets combined by meta-analysis to investigate a potential etiologic relationship between telomere length and RCC risk. We evaluated whether a genetic profile that is associated with longer telomere length is associated with risk of overall RCC and RCC subtypes, and investigated potential modifiers of this relationship.

Material and Methods

The RCC GWAS meta-analysis included a total of 10,784 RCC cases and 20,406 controls of European ancestry from six independent scans conducted at the International Agency for Cancer Research (IARC) (two scans totaling 5,219 RCC cases and 8,011 cancer-free controls; analyzed as a combined dataset), the MD Anderson Cancer Center (MDA) (893 RCC cases, 556 cancer-free controls), the U.S. National Cancer Institute (NCI-1: 1,311 RCC cases, 3,424 cancer-free controls; NCI-2: 2,417 RCC cases, 4,391 cancer-free controls; analyzed separately) and the Institute of Cancer Research (UK) (944 RCC cases, 4,024 cancer-free controls)[25]. Cases were restricted to adults diagnosed with RCC, defined on the basis of the International Classification of Disease for Oncology 2nd and 3rd Edition topography code C64. Samples were genotyped on commercially available Illumina SNP microarrays (HumanHap 300, HumanHap 500, HumanHap 610, HumanHap 660w, HumanHap 1.2M, OmniExpress, Omni5M) after standard quality control metrics. High-quality genotypes were phased and imputation was performed using either MaCH (IARC) or IMPUTE2 (UK, NCI1, NCI2 and UK) with 1000 Genomes Project (Phase 1, Version 3) samples used as a reference panel for imputing missing genotypes. Protocols for studies participating in each GWAS were reviewed by the Institutional Review Boards of their respective institutions. All participants provided written informed consent. Further details on study design and methods have been previously reported[25].

For each study participant, genotypes were extracted for nine previously identified common single nucleotide polymorphisms (SNPs) associated with telomere length in circulating leukocytes (rs10936599, rs11125529, rs2736100, rs3027234, rs6772228, rs755017, rs7675998, rs8105767 and rs9420907). Telomere length associated SNPs not directly genotyped were extracted from imputed data for each scan (**Supplementary Table 1**)[25].

Risk of RCC was evaluated in relation to each of the nine telomere length associated variants. Association testing was conducted separately for each contributing dataset assuming a log-additive (trend) for the effect of the telomere length associated variants on RCC risk. Covariate adjustment differed by dataset and are as follows: 19 significant eigenvectors for IARC, age and two significant eigenvectors for MDA, study indicator variables for NCI1, sex and 3 significant eigenvectors for NCI2, and no covariate adjustment for the UK study. RCC association results for telomere length associated variants from each dataset were combined by meta-analysis using a fixed effects model. Cochran's Q tests for heterogeneity were conducted to identify a lack of consistency across studies.

A GRS was calculated for the nine telomere length associated variants as follows:

$$\mathrm{GRS}_i = \sum_{j=1}^{9} w_j x_{ij}$$

where GRS_i is the risk score for individual i, x_{ij} is the number of telomere length increasing alleles for the jth telomere length associated variant and w_j is the weight or effect coefficient for each telomere length associated variant. A higher GRS value for an individual indicates longer genetically inferred telomere length. Previously published telomere length associated effect estimates (β values) scaled to estimated kilobases of telomere length per length increasing allele were used for w_j[22-24]. GRS association tests were conducted separately for each contributing study using the same covariates as the single SNP association tests previously described. Results from each study were merged by fixed effects meta-analysis and heterogeneity tests were conducted to detect potential departures from homogeneity. Additionally, sub-analyses by RCC subtype as well as analyses stratified by sex, body mass index (BMI), history of hypertension

and smoking status were conducted to comprehensively assess the relationship between telomere length associated variants and RCC risk.

In addition to the GRS analysis, summary statistics from the nine telomere length associated variants were also combined in analyses using an inverse variance weighting method and a likelihood-based method[26]. Both methods use average summary association estimates for the telomere length associated variants with RCC risk to estimate the overall effect of telomere length on RCC risk. These methods produce similar estimates and precision as individual-level data, but have the advantage of using effect statistics from different studies. An online web tool by Burgess et al.[26] accessed at https://sb452.shinyapps.io/summarized/ on February 10, 2017 was used to calculate the inverse variance and likelihood-based estimates. Tests of heterogeneity were performed to assess if a telomere length associated variant's effect on RCC is proportional to its effect on telomere length. Additionally, MR-Egger regression models were fit to evaluate the potential for pleiotropic effects of variants[27].

Unless otherwise stated, statistical analyses and plotting were performed on a 64-bit build of R version 3.3.0 "Supposedly Educational". Meta-analyses were performed using the R package metafor and Egger regression[27] was performed using the R package MendelianRandomization. All statistical tests were two-sided with P values less than 0.05 considered significant.

Results

Associations between the telomere length associated variants and RCC risk are reported in **Table 1** and **Supplementary Figure 1**. Of the nine telomere length associated variants, five variants (rs10936599, rs2736100, rs9420907, rs8105767 and rs6772228) displayed evidence for an individual association with RCC risk ($P<0.05$) and three (rs10936599, rs2736100, rs9420907) were associated at Bonferroni corrected levels ($P<0.006$). This is substantially more than the number of telomere length variants associated with RCC risk that would be expected by chance (exact binomial $P<0.0001$). For all the telomere length-related variants associated with RCC, the allele related to longer telomere length was associated with an increased risk of RCC. There was no evidence for heterogeneity in effect estimates across studies.

We observed a highly statistically significant association between the telomere length GRS and RCC risk (OR=2.07 per predicted kilobase increase, 95% CI=1.70-2.53, $P<0.0001$, **Figure 1**), indicating longer genetically inferred telomere length is associated with increased RCC risk. In an analysis of GRS deciles, a generally monotonic trend across deciles was observed (**Figure 2**). After removing two telomere length variants from the GRS that were in linkage disequilibrium (LD) with RCC susceptibility loci reported in the RCC GWAS (rs10936599 in LD with rs10936602, and rs9420907 in LD with rs11813268; R^2 0.59 and 0.76 in the CEU 1000 Genomes population, respectively[28, 29]), the reduced GRS effect estimate was attenuated but remained statistically significant (OR=1.73 per predicted kilobase increase, 95% CI=1.36-2.21, $P<0.0001$, **Supplementary Figure 2**).

A similar direct relationship between telomere length associated genetic variants and RCC risk was observed when applying summary statistic based approaches to our RCC cases and controls. The likelihood-based pooled estimate for a predicted kilobase increase in telomere

length is a 2.00 increase in the odds of developing RCC (95% CI=1.64-2.43, P<0.0001, **Figure 3**). Likewise, the inverse variance weighted method gave a similar effect estimate (OR=1.96, 95% CI=1.63-2.35, P<0.0001). There was no significant heterogeneity when comparing the ratio of effect sizes of the genetic variants on telomere length to the effect sizes of the genetic variants on RCC risk (P=0.08). Furthermore, results from MR-Egger regression estimated an intercept of -0.043 (95% CI=-0.133 0.047, P=0.4), suggesting no significant evidence for directional pleiotropy (**Supplementary Figure 3**).

In analyses restricted to individual histologic subtypes, comparable associations were observed for each of the telomere length associated variants across RCC subtype (**Supplementary Table 2**). Likewise, similar telomere length associated GRS associations were observed for clear cell RCC (OR=1.93 per predicted kilobase increase, 95% CI=1.50-2.49, P<0.0001, **Supplementary Figure 4**), papillary RCC (OR=1.96, 95% CI=1.01-3.81, P=0.046, **Supplementary Figure 5**) and chromophobe RCC (OR=2.37, 95% CI=0.78-7.17, P=0.13, **Supplementary Figure 6**), although the latter finding did not reach statistical significance. Analyses conducted across strata of sex, BMI, history of hypertension and smoking status did not identify statistically significant evidence of effect modification by these factors (**Supplementary Figures 7–10**).

Discussion

Our findings suggest that an excess of telomere length-related variants is associated with RCC risk and, in aggregate, a genetic risk score predicting longer telomere length in peripheral blood leukocytes is strongly associated with increased RCC risk. The association between longer genetically-predicted telomere length and RCC risk remained statistically significant even after removing two telomere length associated variants highly correlated with GWAS-identified RCC risk variants from the telomere length GRS, indicating additional telomere length associated SNPs are associated with RCC risk beyond these two potentially influential SNPs. We observed no significant differences in the overall telomere length GRS and RCC association across common RCC subtypes, although our power to detect heterogeneity in associations across subtypes was limited. Future studies with larger collections of chromophobe and papillary RCC cases are needed to confirm these associations with telomere length variants by subtype.

With 10,784 RCC cases and 20,406 cancer-free controls, this study is the largest to date to assess the relationship between telomere length and RCC risk. Rather than directly measuring leukocyte telomere length, our study used genetic variants highly associated with leukocyte telomere length as a surrogate of telomere length to assess the relationship with RCC risk. Our genetic approach has several advantages; it is not susceptible to potential biases due to the timing of specimen collection in relation to diagnosis, potential confounding, or differences in pre-analytical specimen processing.

While many lines of evidence in our analysis suggest a clear and robust association between longer telomere length and RCC risk, perhaps the main limitation of our approach is in estimating the magnitude of this association. The telomere length associated variants used in this analysis originated from GWAS studies of leukocyte telomere length, where telomere length was

measured by qPCR[22-24]. These studies then use correlations between qPCR measured telomere length and Southern blot from other laboratories to extrapolate the base pair change in telomere length associated with each variant allele. While these conversions might not be entirely accurate, we chose to use kilobase change in telomere length as weights in our telomere length GRS to facilitate combining variants discovered in different studies into a homogenous telomere length GRS. As such, measurement error may be present in the reported effect estimates; however, the association P values remain valid.

Renal epithelial cell telomere length would perhaps be the best means to assess the relationship between telomere length and RCC risk. Ideally, genetic surrogates of renal epithelial cell telomere length would be available as instruments in our current analysis, but as of publication no genetic variants have been reported to be associated with renal cell telomere length. A prior study has demonstrated that telomere length measurements in leukocytes and non-malignant renal tissue are correlated, with a Pearson correlation coefficient of 0.44[30]. This relationship between leukocyte telomere length and renal cell telomere length suggests the most likely biological mechanism linking increased leukocyte telomere length to RCC risk may be longer correlated renal epithelial cell telomere length. Longer renal telomere length may promote renal tumor growth by increasing replicative potential of renal epithelial cells, although further studies are needed to confirm this hypothesis and alternative explanations are possible. If validated, our findings indicating longer telomere length as a risk factor for RCC may inform clinicians of potential RCC risks associated with administering prolonged treatments with telomerase activating properties (e.g. androgen therapy[31]), particularly in high-risk RCC populations. Additionally, telomere length GRSs, in combination with other genetic, clinical and risk factor data, may hold future clinical value for the development and application of RCC risk

prediction models in support of a “precision prevention” paradigm of targeted disease prevention.

Conclusions

Our investigation adds to the growing body of evidence indicating some aspect of telomere length is important for the development of a variety of common cancer types suggesting clinicians weigh the potential increases in cancer risk when considering treatments with telomerase activating properties. Future studies are needed to decipher which components of telomere biology, whether it be telomere length, telomerase activity or an altogether unknown mechanism, are biologically important in oncogenesis. Such mechanistic insight will lead to improved risk modeling and identify potentially promising targets for drug development.

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The authors declare no relevant conflicts of interest.

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Table and Figure Legends

Table 1. Associations of telomere length associated variants with RCC risk.

Figure 1. Forest plot for associations of the telomere length associated GRS with RCC risk. Odds ratios are scaled to predicted kilobase increase in telomere length. Combined association P<0.0001. Heterogeneity P=0.96.

Figure 2. Associations of telomere length GRS decile with RCC. Dashed line represents the baseline for the reference decile (lowest decile). Error bars represent 95% confidence intervals around the odds ratio association for each GRS decile and RCC.

Figure 3. The effect of each variant on telomere length and RCC risk. Estimates for the SNP--telomere and SNP--RCC associations are presented in Table 1. Error bars around each estimate are 95% confidence intervals around the β estimate. A best fit regression line (dashed line) and 95% confidence interval (shaded region) are plotted using the likelihood based estimate (OR=2.00, 95% CI=1.64-2.43, P<0.0001).