Null Results in Brief

Cancer Epidemiology, Biomarkers & Prevention

Telomere Length and *TERT* Functional Polymorphisms Are Not Associated with Risk of Squamous Cell Carcinoma of the Head and Neck

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Abstract

Background: Recent studies reported associations of the relative telomere length (RTL) and *TERT* variants with risk of several cancers, which have not been comprehensively investigated in squamous cell carcinoma of the head and neck (SCCHN).

Methods: We detected RTL in peripheral blood lymphocytes and genotyped six selected functional single-nucleotide polymorphisms (SNP) of the *TERT* gene in 888 SCCHN cases and 885 cancer-free controls of non-Hispanic whites.

Results: Overall, we did not observe significant associations between RTL and SCCHN risk (adjusted OR = 0.97; 95% CI = 0.80–1.17 for below versus above the median; $P_{trend} = 0.618$) nor between the six *TERT* SNPs and SCCHN risk. We also found no associations between RTL and *TERT* SNPs.

Conclusions: Our results suggest that RTL and *TERT* functional polymorphisms may not play a major role in the etiology of SCCHN. Large prospective studies are needed to validate our findings.

Impact: Although our results suggest no association among RTL, *TERT* functional polymorphisms, and SCCHN risk, this study may contribute to future meta-analysis. *Cancer Epidemiol Biomarkers Prev; 20(12); 2642–5. ©2011 AACR.*

Introduction

Telomeres consist of several thousands (TTAGGG in humans)$_n$ of nucleotide repeats and a protein complex at the ends of chromosomes, maintaining genomic stability by protecting chromosomes from degradation, end to end fusion, and recombination (1). Human telomeres, as a marker for biological age, are approximately 10 to 15 kb in somatic cells and progressively shortened with each cell division. Age-dependent shortening of telomeres impairs function and viability of human cells, and both very short and very long telomeres promote carcinogenesis (2, 3), also a recognized marker carcinogenesis.

TERT encodes the reverse transcriptase component of the telomerase, necessary for the maintenance of telomere length, chromosomal stability, and cellular immortality. Normally, *TERT* mRNA is not expressed in most human somatic cells; however, abnormal expression of *TERT* mRNA and protein occurs in many cancer types, including head and neck cancer. Recently, genome-wide association studies have reported associations between *TERT* genotypes and risk of several cancer types (4, 5), but few studies investigated the correlation among *TERT* genotypes, telomere length, and cancer risk. A recent study showed that *TERT-CLPTM1L* variants were associated with both of the mean relative telomere length (RTL) and cancer risk (4), but another study reported no association of the *TERT-CLPTM1L* rs401681 single-nucleotide polymorphism (SNP) with RTL or cancer risk, including breast cancer, colorectal cancer, and melanoma (6). One study of the FISH-measured RTL suggested a shorter RTL in head and neck cancer but the related risk was not estimated (2), and no study has investigated associations among *TERT* genotypes, RTL, and head and neck cancer risk.

Materials and Methods

The study subjects included 888 non-Hispanic white subjects with newly diagnosed, untreated primary tumors of squamous cell carcinoma of the head and neck (SCCHN), including the oral cavity ($n = 263$; 29.6%), oropharynx ($n = 440$; 49.6%), or larynx and hypopharynx ($n = 185$; 20.8%) recruited between October 1999 and October 2007, who were frequency matched on age, sex, and ethnicity with 885 cancer-free controls identified from hospital visitors at The University of Texas MD Anderson Cancer Center in the same time period. The study design, selection criteria, blood collection, and DNA extraction have been described elsewhere (6).

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doi: 10.1158/1055-9965.EPI-11-0890

We genotyped 6 functional *TERT* SNPs (rs2735940 G > A, rs2736098 C > T, rs2736109 G > A, rs2853669 T > C, rs2853677 A > G, and rs2853690 G >A) using the TaqMan assays with the Sequence Detection Software on an ABI-Prism7900 (Applied Biosystems). These SNPs were chosen because (i) a minor allele frequency of at least 5%, (ii) location in the promoter untranslated region or coding region of the gene, and (iii) previous reports of an association with risk of cancers (4, 5). Primers and probes were supplied by Applied Biosystems. For all genotypes, the assay success rate was more than 99% and the repeated sample's results were 100% concordant. The mean RTL was measured by SYBR Green quantitative real-time PCR as previously described (7). All methods for statistical analysis have been described elsewhere as well (6). Associations of *TERT* genotypes and RTL with SCCHN risk were estimated by computing the OR and their 95% CIs from both univariate and multivariable logistic regression models with or without adjustment for age, sex, smoking, and drinking status. To summarize published case–control association studies of *TERT* polymorphisms and cancer risk, we conducted a meta-analysis. All statistical methods were described elsewhere for association analysis (6) and for meta-analysis (8).

Results

Of the subjects, 37.4% and 49.9% of the 888 cases (aged 56.8 ± 11.3 with 74.8% male) with SCCHN were current smokers and drinkers, respectively, which were higher than that (15.1% and 40.8%) of the 885 cancer-free controls (aged 55.4 ± 11.0 with 74.4% male; P <0.001 for both). The genotype frequencies of the rs2735940 G > A; rs2736098 C > T; rs2736109 G > A; rs2853669 T > C; and rs2853677 A > G SNPs were in agreement with the Hardy–Weinberg equilibrium [P = 0.663, 0.546, 0.530, 0.863, and 0.358, respectively, except for rs2853690 G > A (P = 0.003)]. Overall, no significant associations between these 6 *TERT* SNPs and SCCHN risk were observed after adjustment for age, sex, and smoking, and alcohol status (Table 1).

We then conducted a mini meta-analysis of available from published studies on the association between *TERT* polymorphisms and cancer risk (Fig. 1), and found that, overall, the pooled data showed that *TERT* functional polymorphisms were not significantly associated with cancer risk, except for the rs2736098 (OR = 1.12; 95% CI = 1.06–1.18) for 22,091 cancer cases and 78,540 controls. For RTL, carriers of shorter or longer RTL did not have altered SCCHN risk (adjusted OR = 0.97, 95% CI

Table 1. Genotype frequencies of the *TERT* polymorphisms among SCCHN cases and control subjects and their associations with SCCHN risk

Genotype	Cases n = 888 (%)	Controls n = 885 (%)	Adjusted OR (95% CI)[a]
rs2735940 G > A			
GG	224 (25.2)	221 (25.0)	1.00
GA	440 (49.6)	436 (49.3)	0.99 (0.78–1.26)
AA	224 (25.2)	228 (25.7)	1.03 (0.79–1.36)
rs2736098 C > T			
CC	481 (54.2)	468 (52.9)	1.00
CT	351 (39.5)	356 (40.2)	0.97 (0.79–1.19)
TT	56 (6.3)	61 (6.9)	0.86 (0.58–1.29)
rs2736109 G > A			
GG	319 (35.9)	313 (35.4)	1.00
GA	427 (48.1)	419 (47.3)	1.00 (0.81–1.24)
AA	142 (16.0)	153 (17.3)	0.92 (0.69–1.22)
rs2853669 T > C			
TT	428 (48.2)	425 (48.0)	1.00
TC	381 (42.9)	375 (42.4)	1.02 (0.84–1.26)
CC	79 (8.9)	85 (9.6)	0.91 (0.64–1.29)
ss2853677 A > G			
AA	294 (33.1)	311 (35.1)	1.00
AG	448 (50.5)	416 (47.0)	1.09 (0.88–1.36)
GG	146 (16.4)	158 (17.9)	1.00 (0.75–1.33)
rs2853690 G/A			
GG	596 (67.1)	618 (69.8)	1.00
GA	265 (29.8)	228 (25.8)	1.17 (0.94–1.45)
AA	27 (3.0)	39 (4.4)	0.69 (0.41–1.16)

[a]Adjusted by age, sex, smoking, and drinking status.

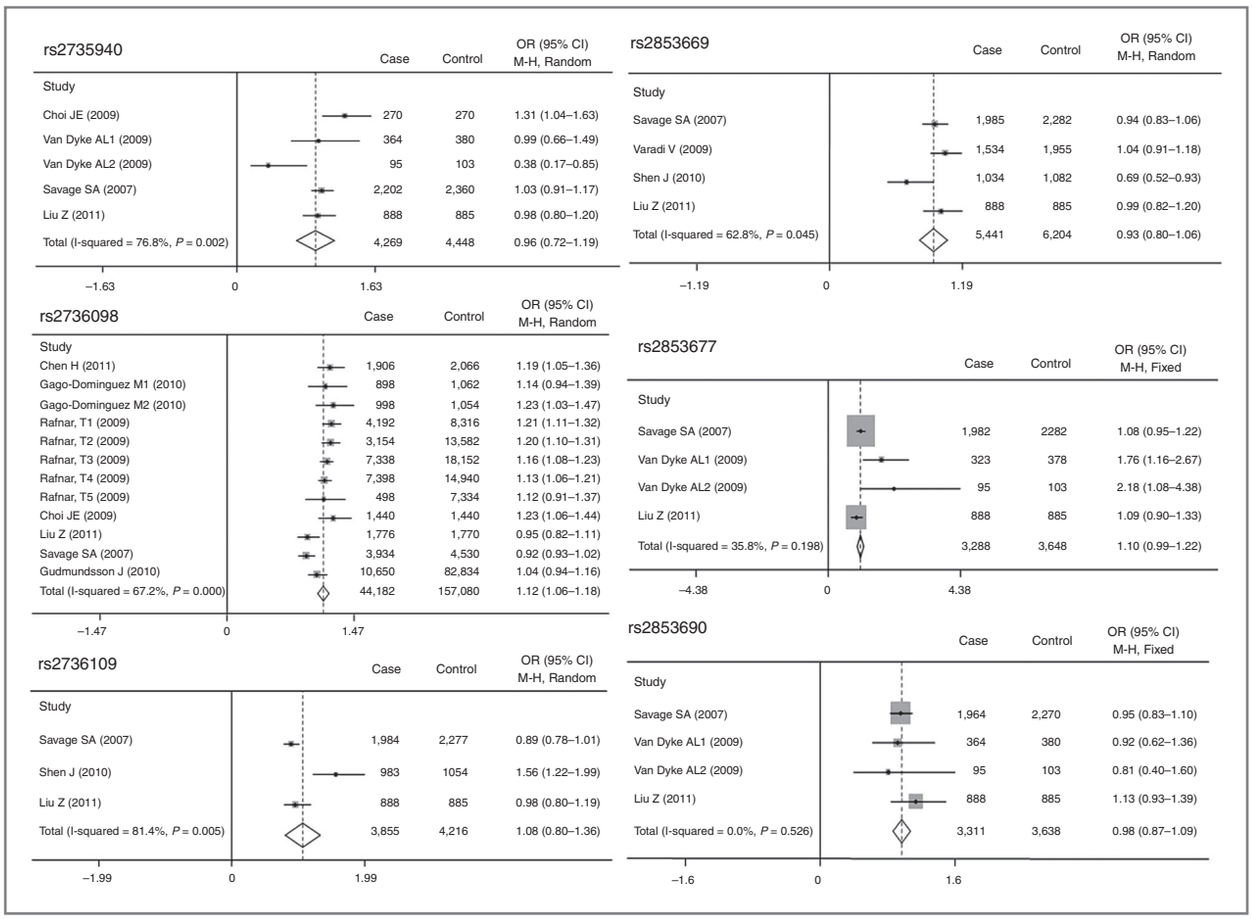

Figure 1. Meta-analysis of associations between 6 SNPs and risk of cancers. OR and 95% CI were calculated using a dominant model for rs2735940, rs2736109, rs2853669, rs2853677, and rs2853690 and an allelic model for rs2736098. Van Dyke AL1 and Van Dyke AL2 represented for lung cancer studies in Caucasian and African Americans, respectively; Gago-Dominguez M1 and Gago-Dominguez M2 represented bladder cancer studies in Caucasian and Chinese, respectively, and Rafnar, T1-5 represented studies for basal cell, lung, bladder, prostate, and cervical cancers, respectively.

= 0.80–1.17), nor was any trend of associations, when RTL was categorized into quartiles ($P_{trend} = 0.618$; Table 2). Finally, we did not find any evidence of associations among the SNPs analyzed, RTL, and SCCHN risk before or after adjustment for age, sex, smoking, and drinking status (data not shown).

Discussion

In this study of associations among RTL, *TERT* SNPs, and SCCHN risk, the largest of all published case–control studies, only next to a recent breast cancer study (8), we found no evidence of associations in 888 SCCHN patients and 885 cancer-free controls in a non-Hispanic white population. This finding is consistent with our mini meta-analysis results. Our study sample size had a statistical power of 80% to detect an OR of 0.611 or 1.508 with an average *TERT* risk genotype of 10% or to detect a difference in RTL as small as 0.604 using our observations in this study, compared with the reported 0.9 (±0.2) from the only one published study of 92 head and neck cancer cases and 92 controls (2).

The *TERT* gene has been identified as a catalytic subunit and a key regulator of telomerase activity, and overexpression of *TERT* is thought to be involved in the tumorigenesis of various cancers, including SCCHN. Although several association studies evaluated the role of *TERT* polymorphisms in cancer risk, the results are inconclusive, mostly because of small samples included in the published studies. One recent large case–control study did not find an association of *TERT* SNP (rs401681) with risk of cancers of the breasts, colorectum, and skin, nor with telomere length (7). Telomere length in blood lymphocytes is considered a tumor marker (2, 3), but the results from association studies are also inconclusive in some cancers. One recent prospective study of breast cancer failed to validate the findings from previous large case–control study carried out by the same group

Table 2. Comparison and association of the RTLs between patients with SCCHN and cancer-free controls

RTL [Relative Telomere Length]	Cases		Controls		*P*	Crude OR (95% CI)	Adjusted OR (95% CI)[a]
	n (%)	Mean ± SD	*n* (%)	Mean ± SD			
Overall	888 (100)	2.07 ± 5.05	885 (100)	1.90 ± 4.53	0.460[a]		
By median							
≥1.09	467 (52.6)	3.59 ± 6.59	445 (50.3)	3.40 ± 6.01	0.651[b]	1.00	1.00
<1.09	421 (47.4)	0.38 ± 0.33	440 (49.7)	0.38 ± 0.34	0.987[b]	0.91 (0.76–1.10)	0.97 (0.80–1.17)
By quartile							
4th (≥2.28)[c]	230 (25.9)	5.64 ± 8.95	219 (24.8)	4.16 ± 8.18	0.631[b]	1.00	1.00
3rd (1.09–2.28)	237 (26.7)	1.61 ± 0.34	226 (25.5)	1.62 ± 0.35	0.824[b]	1.00 (0.77–1.30)	1.00 (0.76–1.31)
2nd (0.25–1.09)	219 (24.7)	0.63 ± 0.26	217 (24.5)	0.67 ± 0.25	0.134[b]	0.96 (0.74–1.25)	1.01 (0.77–1.33)
1st (<0.25)[d]	202 (22.8)	0.09 ± 0.08	223 (25.2)	0.09 ± 0.06	0.308[b]	0.86 (0.66–1.13)	0.92 (0.70–1.22)

[a]Two-sided Student *t* tests for differences between cases and controls.
[b]Adjusted by age, sex, smoking, and drinking status.
[c]Fourth quartile represents longest quartile of RTL.
[d]First quartile represents shortest quartile of RTL.

(9), similarly as we found in our recent meta-analysis of 11,255 cases and 13,101 controls from 21 publications (8), namely, the case–control findings were not confirmed by prospective studies, suggesting that single larger, well-design prospective studies are warranted to confirm the reported findings.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors thank Margaret Lung and Kathryn L. Tipton for their assistance in recruiting the subjects, Min Zhao, Hongping Yu, Jianzhong He, and Kejing Xu, for their laboratory assistance.

Grant Support

This work was supported by NIH grants R01 ES 11740-07 and CA 131274-01 (to Q. Wei) and CA 16672 (to MD Anderson Cancer Center).

Received September 22, 2011; accepted October 3, 2011; published OnlineFirst October 12, 2011.

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