Association of Leukocyte Telomere Length and Genes Involved in its Regulation With Oral Carcinoma

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Abstract. *Background/Aim: This study aimed to determine the relationship between the relative leukocyte telomere length (RLTL) and gene polymorphisms involved in its regulation with the occurrence of oral squamous cell carcinoma (OSCC). Patients and Methods: Patients with OSCC and healthy subjects were examined. Genotyping and RLTL measurement were carried out using rPCR. Results: The OSCC group had longer telomeres than controls (p=0.001). Minor allele T at TERF1rs1545827 may increase RLTL shortening (p=0.047). TNKS2rs10509639 A/G and A/G+G/G genotypes were associated with a 2.6-fold increased odd (p=0.012) and a 2.4-fold increased odd (p=0.019) of RLTL elongation compared to A/A genotype. The A/G genotype was associated with a 2.6-fold increased odd (p=0.011) compared to the A/A+G/G genotypes. Each G allele was associated with a 2.1-fold increased odd of longer RLTL (p=0.036). Conclusion: Longer telomeres were found in patients with OSCC than in controls. The TERF1 rs1545827 and the TNKS2 rs10509639 polymorphisms were associated with an increase in RLTL.*

Oral squamous cell carcinoma (OSCC) accounts for 20% of all head and neck squamous cell carcinoma (HNSCC) cases worldwide, which makes it one of the most common types of malignant tumors of this anatomical region. This broad

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prevalence can be associated with risk factors, such as the use of tobacco products and alcohol consumption that are also associated with the development and progression of various chronic diseases. Early cancer diagnosis (stage I-II), rises the patient survival rate up to 70-90%. For this purpose, new molecular biological markers could be of service in diagnosing the disease at its early stages (1). Molecular markers are indicators of normal biological, pathological and pharmacological molecular responses, which can provide useful information for diagnosis and prognosis (2).

Lately, the attention has been focused on telomeres and telomere length regulating genes, due to their importance in aging and the development of chronic diseases and malignant tumors. The name telomere itself originates from the Greek word "telos", which means the end, and "meros", which means a part (3). The main function of telomeres is to protect the ends of chromosomes from degradation and end-to-end fusions (4). There is also a telomere protein complex, called shelterin, that is incredibly important for the protection of the ends of chromosomes and the regulation of telomere length (5). Although telomeres in the human generative cells and embryotic stem cells are kept active by the help of telomerases, telomeres become shorter when somatic cells divide, because of insufficient telomerase expression (4). There is a significant number of genes and their coding proteins that are associated with the regulation of telomeres. TRF1 and TRF2 are negative telomere length regulators – telomerase inhibitors, which means that overexpression of TRF1 and TRF2 causes shortening of telomeres (6, 7). Tankirase 2 (TNKS2) protects the linear chromosome structure (8). TERT gene encodes the catalytic subunit of telomerase and plays a major role in telomere DNA length preservation and carcinogenesis (9). Telomerase is a ribonucleic acid-protein complex, which is responsible for maintaining the length of telomeres (10). It is highly active

Table I. *Demographic characteristics.*

	Control group	OSCC	*p* mean
Age, Mean (SD)	62.02 (11.15)	59.49 (11.32)	0.163*
Gender, N, (%)			0.368**
Male	144 (70.6)	35 (74.5)	
Female	60 (29.4)	12 (25.5)	
Stage, N (%)			
I	-	2 (4,3)	-
II		13 (27.7)	
III		12 (25.5)	
IV		20 (42.6)	
G Status, N (%)			
1		1 (2.2)	
2	-	36 (78.3)	-
3		9 (19.6)	

OSCC: Oral squamous cell carcinoma; SD: standard deviation; *Student's *t*-test; **Fisher's Exact test; G status: tumor differentiation grade.

Table II. *Relationship between OSCC stage and relative leukocyte telomere length.*

	OSCC stage				*p*-Value*
	I	II	III	IV	
RLTL; median (IQR)	0.560 (-)	1.719 (5.273)	1.505 (3.424)	1.952 (3.573)	0.047**

IQR: Interquartile range; OSCC: oral squamous cell carcinoma; *Mann-Whitney *U*-test; **I stage *vs*. IV stage.

Table III. *Relationship between OSCC G status (tumor differentiation grade) and relative telomere length.*

	OSCC G status			*p*-Value*
	1	2	3	
RLTL; median (IQR)	-	1.432 (3.574)	1.500 (2.926)	0.872**

IQR: Interquartile range; *Mann-Whitney *U*-test; **G2 *vs*. G3.

in cancer cells, resulting in unlimited replication potential and growth of the tumor (11).

It is particularly important to understand the mechanisms regulating telomere length and the relation between telomere length changes and various diseases. Discovery of new molecular markers is required to diagnose tumorous diseases, identify the risk groups for disease development, predict the response to treatment and carry out patients' observations in order to apply personalized treatment. For this reason, we assessed the associations of *TERT* rs2736098, *TERT-CLPTM1* rs401681, *TRF1* rs1545827, rs10107605, *TNKS2* rs10509639 and rs10509637, and *TERF2* rs251796 gene polymorphisms and telomere length with OSCC.

Materials and Methods

All the procedures used in this study were approved by the Kaunas Regional Ethics Committee for Biomedical Research, Lithuania in compliance with ethical standards (permission number is BE-2-34). The study was conducted at the Department of Otorhinolaryngology, and Ophthalmology Laboratory of Neuroscience Institute, Lithuanian University of Health Sciences, Kaunas, Lithuania.

Study group. The current study included 47 patients (35 males and 12 females with a mean age of 59.5 years old) with a diagnosis of OSCC and 204 healthy control group subjects (144 males and 60 females, with a mean age of 62.02 years old) (Table I).

The patients included in the study had OSCC with no additional diseases, their general health status was good, and they had provided a written informed consent for participation in the study. The final diagnosis of OSCC was confirmed following histopathological investigation after a biopsy or surgery. Subjects chosen to participate as a control group did not have any oncological diseases, their general health status was good, and they provided a written informed consent for inclusion in the study.

DNA extraction, genotyping, relative leukocyte telomere length measurement and statistical analysis. DNA extraction, genotyping and measurement of the relative leukocyte telomere length (RLTL) methods, as well as statistical analysis, have been described in detail in our previous studies (12, 13).

Results

OSCC patients had statistically significantly longer telomeres than healthy controls [median (IQR): 1.5 (3.34) *vs*. 0.95 (1.43)], *p*=0.001, respectively. Results are shown in Figure 1.

After analyzing patients' relative leukocyte telomere length in relation to the stage of the disease, we determined that OSCC patients with stage IV cancer had longer relative leukocyte telomere length than those with stage I [median (IQR): 1.952 (3.573) *vs*. 0.560 (-), *p*=0.047] (Table II). Analogous analysis was performed with different G status (tumor differentiation grade) groups of OSCC patients, but no statistically significant results were acquired (Table III).

We also determined statistically significant differences between telomere lengths of healthy control group individuals and OSCC patients with different stages of the disease (control group stage is marked with 0). OSCC patients with stage II and IV disease had longer relative leukocyte telomere length than controls (*p*=0.014 and *p*=0.003, respectively). Results are shown in Table IV.

TERT rs2736098, *TERT-CLPTM1* rs401681, *TRF1* rs1545827, rs10107605, *TNKS2* rs10509639 and rs10509637,
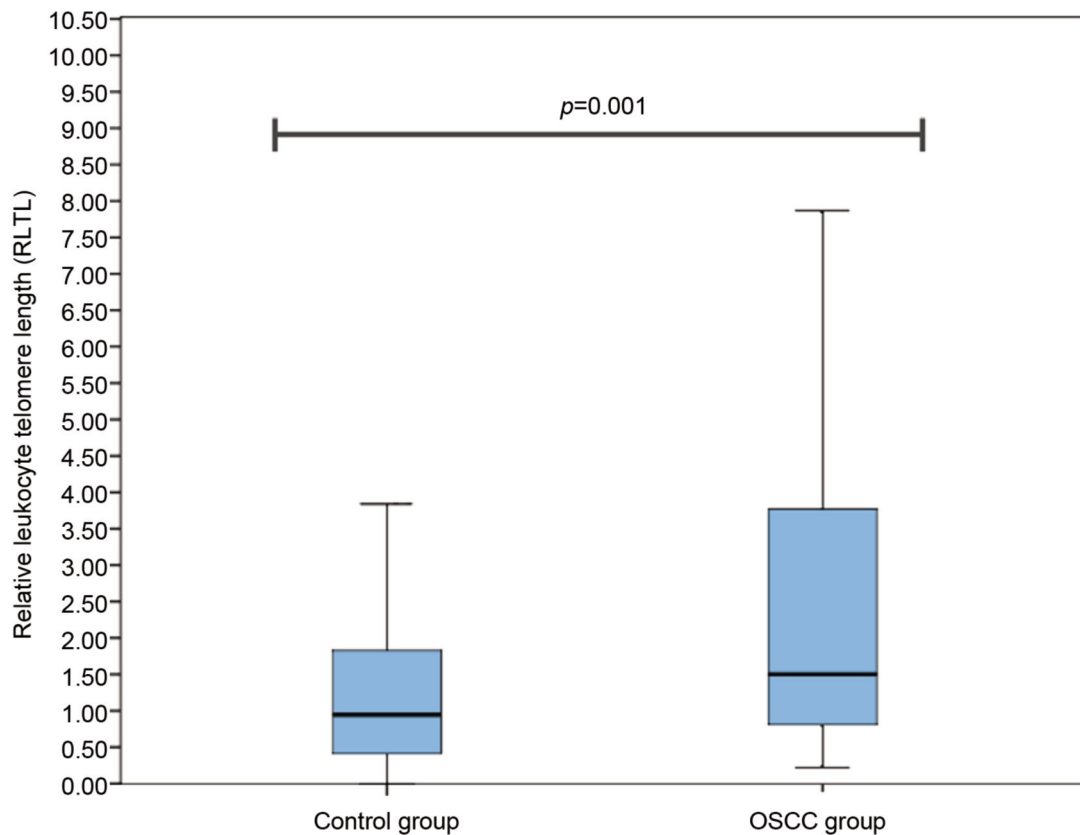
Figure 1. *Relative leucocyte telomere length in the control and OSCC groups. Relative leukocyte telomere length (RLTL) in OSCC group versus control group are presented as box-and-whisker plots with the median and interquartile range. Mann-Whitney U-test was used to assess the differences of RLTL between OSCC and control groups.*

TERF2 rs251796 matched the Hardy-Wainber equilibrium (HWE) (*p*>0.001) (Table V). After analyzing these SNPs' distribution, no statistically significant differences were found between the OSCC patients and control groups (Table VI).

Analysis of the SNPs (*TERT* rs2736098, *TERT-CLPTM1* rs401681, *TERF1* rs1545827, rs10107605, *TNKS2* rs10509639 and rs10509637, *TERF2* rs251796) and binary logistic regression association with the age and gender of patients with OSCC and healthy controls, did not reveal any statistically significant differences.

The relative leukocyte telomere length of the healthy control group individuals and OSCC patients was analyzed in relation to the SNPs (*TERT* rs2736098, *TERT-CLPTM1* rs401681, *TERF1* rs1545827, rs10107605, *TNKS2* rs10509639 and rs10509637, *TERF2* rs251796). This analysis showed no statistically significant results (Table VII). We also searched for an association between the control group subjects and OSCC patients' leukocyte telomere length and SNP genotypes. Analysis showed that at least one rare allele at *TERF1,* rs1545827, increased the possibility of telomere shortening (*p*=0.047) in the healthy control group. Analysis

Table IV. *The comparison of relative leukocyte telomere length differences between four OSCC stages and the control group.*

Stage	*p*-Value*
0 *vs.* I	0.418
0 *vs.* II	0.014
0 *vs.* III	0.246
0 *vs.* IV	0.003

*Mann-Whitney *U*-test.

of the other polymorphisms showed no statistically significant results. Results are given in Table VIII and Table IX.

For further analysis, subjects were divided in two groups according to telomere length: short and long telomere groups. The *TNKS2* rs10509639 genotype rate was found to be statistically significantly different between the short and long telomere groups (*p*=0.022). Also, rs10509639 G allele was more common in subjects who had long telomeres (*p*=0.036) (Table X).

Table V. *Hardy-Weinberg equilibrium analysis.*

SNP	Allele rate	Gene distribution	HWE *p*-Value
TERT rs2736098	C 0.73	17/77/110	0.50
	T 0.27		
TERT rs401681	C 0.62		
	T 0.38	26/105/73	0.21
TERF1 rs1545827	C 0.59		
	T 0.41	32/105/67	0.39
TERF1 rs10107605	A 0.87		
	C 0.13	6/41/157	0.11
TNKS2 rs10509637	A 0.82		
	G 0.18	8/59/137	0.61
TNKS2 rs10509639	A 0.92		
	G 0.08	0/32/172	0.22
TERF2 rs251796	A 0.71		
	G 0.29	22/76/106	0.14

SNP: Single-nucleotide polymorphism.

Binary logistic analysis was performed in order to evaluate the effect of *TERT* rs2736098, *TERT-CLPTM1* rs401681, *TERF1* rs1545827, rs10107605, *TNKS2* rs10509639 and rs10509637, *TERF2* rs251796 on the length of telomeres. Based on the analysis, the *TNKS2* rs10509639 polymorphism A/G genotype and the A/G and G/G genotypes together increased the odd of telomere lengthening 2.0-fold (OR=2.555; 95%CI=1.227-5.318; *p*=0.012), and 2.4-fold (OR=2.358; 95%CI=1.152-4.827; *p*=0.019), respectively, in comparison to A/A genotype. Also, the odds ratio of telomere length was increased 2.6-fold by the A/G genotype, in comparison to A/A and G/G genotypes together (OR=2.578; 95%CI=1.239-5.366; *p*=0.011) and each G allele increased the odd 2.1-fold (OR=2.090; 95%CI=1.050-4.160; *p*=0.036) (Table XI).

Discussion

Despite the development of modern medical technologies and the increasing number of diagnostic methods, the survival rate of patients with advanced (III-IV stage) HNSCC, as well as with OSCC, has increased only up to 65% (14). In order to increase the survival rate of patients, research is focused on identifying new markers such as telomere length and gene polymorphisms regulating telomere length (TERT rs2736098, TERT-CLPTM1 rs401681, TRF1 rs1545827, rs10107605, TNKS2 rs10509639 and rs10509637, TERF2 rs251796), which could be useful for diagnosis and treatment.

Results from analyses of telomere length in cancer patients are scarce and inconsistent. Our results revealed that OSCC patients had statistically significantly longer telomeres than healthy controls [median (IQR): 1.5 (3.34) *vs*. 0.95 (1.43),

Table VI. *TERT rs2736098, TERT-CLPTM1 rs401681, TRF1 rs1545827, rs10107605, TNKS2 rs10509639 and rs10509637, TERF2 rs251796 genotype polymorphisms and alleles' frequencies in OSCC patients and control group individuals.*

SNP	Genotype	Control group N (%)	Floor of the mouth cancer N (%)	*p*-Value*
TERT rs2736098	CC	110 (53.9)	24 (51.1)	0.936
	CT	77 (37.7)	19 (40.4)	
	TT	17 (8.3)	4 (8.5)	
	Alleles	0.766		
	C	297 (72.8)	67 (71.3)	
	T	111 (27.2)	27 (28.7)	
TERT-CLPTM1 rs401681	CC	73 (35.8)	19 (40.4)	0.453
	CT	105 (51.5)	25 (53.2)	
	TT	26 (12.7)	3 (6.4)	
	Alleles	0.320		
	C	251 (61.5)	63 (67.0)	
	T	157 (38.5)	31 (33)	
TERF1 rs1545827	CC	67 (32.8)	11 (23.4)	0.447
	CT	105 (51.5)	28 (59.6)	
	TT	32 (15.7)	8 (17.0)	
	Alleles			
	C	239 (58.6)	50 (53.2)	0.341
	T	169 (41.4)	44 (46.8)	
TERF1 rs10107605	AA	157 (77.0)	38 (80.9)	0.479
	AC	41 (20.1)	9 (19.1)	
	CC	6 (2.9)	0 (0.0)	
	Alleles			
	A	355 (87.0)	85 (90.4)	0.364
	C	53 (13.0)	9 (9.6)	
TNKS2 rs10509637	AA	137 (67.2)	34 (72.3)	0.749
	AG	59 (28.9)	11 (23.4)	
	GG	8 (3.9)	2 (4.3)	
	Alleles			
	A	333 (81.6)	79 (84.0)	0.581
	G	75 (18.4)	15 (16.0)	
TNKS2 rs10509639	AA	172 (84.3)	36 (76.6)	0.070
	AG	32 (15.7)	10 (21.3)	
	GG	0 (0.0)	1 (2.1)	
	Alleles			
	A	376 (92.2)	82 (87.2)	0.128
	G	32 (7.8)	12 (12.8)	
TERF2 rs251796	AA	106 (52.0)	22 (46.8)	0.805
	AG	76 (37.3)	19 (40.4)	
	GG	22 (10.8)	6 (12.8)	
	Alleles			
	A	288 (70.6)	63 (67.0)	0.497
	G	120 (29.4)	31 (33.0)	

SNP: Single-nucleotide polymorphism; *Pearson's χ^2 test.

p=0.001, respectively]. A number of studies have examined associations between telomere length and cancer, but only few studies have focused on head and neck cancer, telomere length and telomere length regulating gene polymorphisms. Zhang *et al.* have demonstrated that short telomere length in peripheral blood mononuclear cells (PBMCs) was strongly associated

Table VII. *Association between relative leukocyte telomere length and SNP genotypes.*

SNP	22 Median (IQR)	11+12 Median (IQR)	*p*-Value*	12+22 Median (IQR)	11 Median (IQR)	*p*-Value*
TERT rs2736098	1.176 (2.05)	0.997 (1.51)	0.313	1.11 (1.85)	0.964 (1.27)	0.297
TERT-CLPTM1 rs401681	0.883 (1.19)	1.012 (1.82)	0.224	1.052 (1.54)	0.881 (1.68)	0.294
TRF1 rs1545827	1.007 (1.25)	0.997 (1.63)	0.6	0.933 (1.44)	1.057 (1.82)	0.061
TRF1 rs10107605	0.745 (1.58)	1.017 (1.59)	0.258	0.965 (1.13)	1.007 (1.81)	0.497
TNKS2 rs10509637	1.298 (1.52)	0.99 (1.57)	0.458	1.010 (2.04)	1.003 (1.39)	0.557
TNKS2 rs10509639	-	1.005 (1.6)	-	1.275 (1.56)	0.954 (1.61)	0.187
TERF2 rs251796	0.857 (1.81)	1.028 (1.6)	0.668	0.94 (1.61)	1.112(1.59)	0.588

11-homozygotes, who have the more frequent allele, 12-heterozygotes, 22-homozygotes, who have the rarer allele; SNP: single-nucleotide polymorphism; *Mann-Whitney *U*-test.

Table VIII. *Association between healthy control group individuals' relative leukocyte telomere length and SNP genotypes.*

SNP	22 Median (IQR)	11+12 Median (IQR)	*p*-Value*	12+22 Median (IQR)	11 Median (IQR)	*p*-Value*
TERT rs2736098	0.878 (1.9)	0.959 (1.39)	0.709	0.967 (1.84)	0.933 (1.2)	0.453
TERT-CLPTM1 rs401681	0.937 (1.16)	0.947 (1.62)	0.596	1.003 (1.37)	0.858 (1.72)	0.478
TRF1 rs1545827	0.832 (1.43)	0.957 (1.5)	0.552	0.825 (1.29)	1.043 (1.75)	0.047
TRF1 rs10107605	0.745 (1.58)	0.957 (1.44)	0.347	0.954 (1.19)	0.94 (1.57)	0.781
TNKS2 rs10509637	1.175 (1.51)	0.933 (1.43)	0.84	0.883 (1.66)	0.959 (1.33)	0.818
TNKS2 rs10509639	-	0.947 (1.43)	-	1.233 (1.58)	0.884 (1.34)	0.246
TERF2 rs251796	0.731 (1.61)	0.963 (1.42)	0.75	0.88 (1.41)	1.005 (1.54)	0.714

11-homozygotes, who have the more frequent allele, 12-heterozygotes, 22-homozygotes, who have the rarer allele; SNP: single nucleotide polymorphism; *Mann-Whitney *U*-test.

Table IX. *Association between relative leukocyte telomere length and SNP genotypes in OSCC patients.*

SNP	22 Median (IQR)	11+12 Median (IQR)	*p*-Value*	12+22 Median (IQR)	11 Median (IQR)	*p*-Value*
TERT rs2736098	4.172 (5.1)	1.479 (3)	0.159	1.952 (4.09)	1.432 (2.07)	0.448
TERT-CLPTM1 rs401681	0.693 (-)	1.562 (3.76)	0.147	1.782 (4.9)	1.21 (1.67)	0.095
TRF1 rs1545827	1.21(4.58)	1.782 (3.21)	0.773	1.5 (3.78)	2.014 (3.09)	0.56
TRF1 rs10107605	-	1.5 (3.34)	-	1.21 (2.01)	1.782 (4.26)	0.385
TNKS2 rs10509637	4.862 (-)	1.479 (3.02)	0.22	3.531 (4.2)	1.277 (2.17)	0.147
TNKS2 rs10509639	-	1.562 (3.53)	-	1.624 (2.42)	1.5 (3.96)	0.91
TERF2 rs251796	1.103 (4.27)	1.625 (3.67)	0.577	1.31 (4.12)	1.562 (2.24)	0.715

11-homozygotes, who have the more frequent allele, 12-heterozygotes, 22-homozygotes, who have the rarer allele; SNP: single-nucleotide polymorphism; *Mann-Whitney *U*-test.

with a moderately increased risk of oropharyngeal squamous cell carcinoma; however, not with an increased risk of oral cavity cancer. Associations between telomere length and a higher risk of HPV16-positive oropharyngeal carcinoma and tumor HPV16 status have also been revealed (15). Bau *et al*. have observed that short leukocyte telomere length was associated with an increased risk of developing oral premalignant lesions and precursors of OSCC (16). A study performed by Alves-Paiva *et al*. has also found that RLTL was significantly shorter in patients with head and neck cancer (HNC) in comparison to healthy controls (*p*=0.0003). Patients with shortest RLTL had an increased risk of developing HNC (*p*<0.0001). No significant correlation was observed between RLTL and patients' clinical features and personal habits (17).

Table X. *Relative telomere length and SNP association.*

SNP	Genotype	Short telomeres N (%)	Long telomeres N (%)	p-Value*
TERT	CC	68 (55.7)	64 (52.0)	0.844
rs2736098	CT	44 (36.1)	48 (39.0)	
	TT	10 (8.2)	11 (8.9)	
	Alleles			
	C	180 (73.8)	176 (71.5)	0.580
	T	64 (26.2)	70 (28.5)	
TERT-CLPTM1	CC	50 (41.0)	40 (32.5)	0.226
rs401681	CT	56 (45.9)	70 (56.9)	
	TT	16 (13.1)	13 (10.6)	
	Alleles			
	C	156 (63.9)	150 (61.0)	0.499
	T	88 (36.1)	96 (39.0)	
TERF1	CC	33 (27.0)	44 (35.8)	0.282
rs1545827	CT	70 (57.4)	59 (48.0)	
	TT	19 (15.6)	20 (16.3)	
	Alleles			
	C	136 (55.7)	147 (59.8)	0.368
	T	108 (44.3)	99 (40.2)	
TERF1	AA	94 (77.0)	97 (78.9)	0.247
rs10107605	AC	23 (18.9)	25 (20.3)	
	CC	5 (4.1)	1 (0.8)	
	Alleles			
	A	211 (86.5)	219 (89.0)	0.389
	C	33 (13.5)	27 (11.0)	
TNKS2	AA	83 (68.0)	84 (68.3)	0.399
rs10509637	AG	36 (29.5)	32 (26.0)	
	GG	3 (2.5)	7 (5.7)	
	Alleles			
	A	202 (82.8)	200 (81.3)	0.668
	G	42 (17.2)	46 (18.7)	
TNKS2	AA	109 (89.3)	96 (78.0)	0.022
rs10509639	AG	12 (9.8)	27 (22.0)	
	GG	1 (0.8)	0 (0)	
	Alleles			
	A	230 (94.3)	219 (89.0)	0.036
	G	14 (5.7)	27 (11.0)	
TERF2	AA	56 (45.9)	70 (56.9)	0.185
rs251796	AG	49 (40.2)	42 (34.1)	
	GG	17 (13.9)	11 (8.9)	
	Alleles			
	A	161 (66.0)	182 (74.0)	0.053
	G	83 (34.0)	64 (26.0)	

SNP: Single nucleotide polymorphism.

Table XI. *TERT rs2736098, TERT-CLPTM1 rs401681, TRF1 rs1545827, rs10107605, TNKS2 rs10509639 and rs10509637, TERF2 rs251796 binary logistic regression analysis.*

Model	Genotype/ Allele	OR (95%CI)	p-Value	AIC
TERT rs2736098				
Co-dominant	C/T	1.159 (0.680-1.975)	0.587	343.299
	T/T	1169 (0.465-2.938)	0.740	
Dominant	C/T+T/T	1161 (0.702-1.919)	0.561	341.300
Recessive	T/T	1.100 (0.449-2.693)	0.835	341.594
Over-dominant	C/T	1.135 (0.676-1.904)	0.633	341.409
Additive	T	1.112 (0.755-1.638)	0.591	341.348
TERT rs401681				
Co-dominant	C/T	1.562 (0.907-2.693)	0.108	340.659
	T/T	1.016 (0.438-2.357)	0.971	
Dominant	C/T+T/T	1.441 (0.855-2.429)	0.170	339.747
Recessive	T/T	0.783 (0.359-1.706)	0.538	341.257
Over-dominant	C/T	1.557 (0.940-2.577)	0.085	338.660
Additive	T	1.150 (0.782-1.691)	0.477	341.131
TERF1 rs1545827				
Co-dominant	C/T	0.632 (0.358-1.117)	0.114	341.101
	T/T	0.789 (0.364-1.711)	0.549	
Dominant	C/T+T/T	0.666 (0.387-1.147)	0.142	339.470
Recessive	T/T	1.053 (0.531-2.088)	0.883	341.616
Over-dominant	C/T	0.685 (0.414-1.133)	0.141	339.459
Additive	T	0.836 (0.574-1.216)	0.349	340.757
TERF1 rs10107605				
Co-dominant	A/C	1.053 (0.559-1.984)	0.872	340.601
	C/C	0.194 (0.022-1.690)	0.138	
Dominant	A/C+C/C	0.900 (0.492-1.647)	0.732	341.521
Recessive	C/C	0.192 (0.022-1.666)	0.134	338.626
Over-dominant	A/C	1.098 (0.584-2.065)	0.772	341.554
Additive	C	0.803 (0.477-1.353)	0.411	340.956
TNKS2 rs10509637				
Co-dominant	A/G	0.878 (0.499-1.545)	0.652	341.755
	G/G	2.306 (0.576-9.220)	0.238	
Dominant	A/G+G/G	0.988 (0.577-1.692)	0.965	341.636
Recessive	G/G	2.394 (0.604-9.481)	0.214	339.958
Over-dominant	A/G	0.840 (0.480-1.471)	0.542	341.265
Additive	G	1.100 (0.702-1.724)	0.677	341.464
TNKS2 rs10509639				
Co-dominant	A/G	2.555 (1.227-5.318)	0.012	335.510
	G/G	-	1.000	
Dominant	A/G+G/G	2.358 (1.152-4.827)	0.019	335.812
Recessive	G/G	-	1.000	340.239
Over-dominant	A/G	2.578 (1.239-5.366)	0.011	335.769
Additive	G	2.090 (1.050-4.160)	0.036	336.996
TERF2 rs251796				
Co-dominant	A/G	0.686 (0.399-1.179)	0.172	340.249
	G/G	0.518 (0.224-1.194)	0.123	
Dominant	A/G+G/G	0.642 (0.388-1.064)	0.085	338.660
Recessive	G/G	0.607 (0.272-1.355)	0.223	340.121
Over-dominant	A/G	0.772 (0.459-1.299)	0.330	340.687
Additive	G	0.708 (0.488-1.027)	0.069	338.274

OR: Odds ratio; CI: confidence interval; AIC: Akaike information criterion.

The opposite results were revealed by Liu *et al*. who have reported that relative telomere length may be not important in HNSCC carcinogenesis (18). Oh *et al*., have suggested that the advancement of tumors may correlate with long telomere length and a poor prognosis (19). The authors stated that increased telomere length indicates that telomere maintenance may also be important for the progression of

HNSCC (20). Our results are inconsistent with previous studies, except for the study by Oh *et al*. Also, longer telomeres can increase the possibility of cancer development by stimulating cell immortality, and causing genome instability; the association between long telomeres and an advanced stage with lower survival rate has been found in colorectal and liver carcinoma patients (19, 21, 22).

Therefore, there are many studies suggesting that RLTL is associated with other types of cancer, and that both short and long telomeres can be associated with cancer development (23-27). However, a big meta-analysis performed by Weischer *et al*. has shown that RLTL is not associated with cancer risk (25). Da-Tian Bau *et al*. have stated that different cancers have different etiology, and pooling all cancer types together may mask the significant associations of RLTL with individual cancer types (16).

In our study, the *TERF1* rs1545827 polymorphism was associated with an increased RLTL while the *TNKS2* rs10509639 polymorphism was associated with longer RLTL. Ying Bao *et al*. have found that the *TERT* rs401681 rarer allele (A) was statistically significantly associated with telomere shortening (*p*=0.023) in pancreatic cancer patients (28). Carriers of the C allele of the rs2736100 polymorphism were statistically significantly associated with longer telomeres and a higher risk of cancer, while the A allele carriers were associated with shorter telomeres (29). *TERT* rs2736098 polymorphism increases the risk of lung, heart, bladder cancer and other malignancies (30). Also, Liu *et al*. have found that *TERT* rs2736098 with *TERT-CLPTM1* rs401681 genotype variants are statistically significantly associated with reduced chances of having HNSCC (*p*=0.048) (31). *TERF2* rs251796 G variant increases the risk for lung cancer (*p*=0.03) (32). The *TNKS2* rs10509637 rare allele G was associated with an increased risk of breast cancer (*p*=0.006) (33). Furthermore, several studies have examined the association of single nucleotide polymorphisms with telomere regulation. For example, in a metanalysis performed in 2013, Bojesen *et al*. have found that the *TERT* gene rs7705526 rarer allele showed statistically significant association with longer telomeres (*p*=2.3×10^{-14}) and increases the possibility of low malignancy ovarian cancer (*p*=1.3×10^{-15}) (34). The results of other metanalyses showed that the *TERT* gene rs7726159 rarer allele (A), showed statistically significant associations with glioma and lung cancer (*p*=4.38×10^{-36}), but negative associations with testicular cancer, prostate cancer and pancreatic cancer (*p*=5.07×10^{-6}) (35). Research on *TNKS2* rs1340420 showed that the G/G and A/G genotypes reduced the risk of lung adenocarcinoma (*p*=0.03); whereas the *TNKS2* rs1770474 rarer (T) allele increased the risk of lung squamous cell carcinoma in women 4.7 times (*p*=0.049) (36).

In order to evaluate *TERT* rs2736098, *TERT-CLPTM1* rs401681, *TRF1* rs1545827, rs10107605, *TNKS2* rs10509639 and rs10509637, *TERF2* rs251796 polymorphisms' association with OSCC and telomere length changes, more subjects have to be analysed.

Conclusion

The relative telomere length was significantly higher in the group of OSCC patients compared to healthy controls. In OSCC patients, the *TERF1* rs1545827 and *TNKS2* rs10509639 polymorphisms were associated with increased relative telomere length.

Conflicts of Interest

None of the Authors have any conflicts of interest related to this submission.

Authors' Contributions

R.L., A.V., G.G., A.K., A.S., and V.U. designed research; Z.J., R.L., A.V., G.G., A.K., and V.U. performed research; A.V., G.G., Z.J., and V.U. analyzed data; and R.L., A.V., G.G., A.K., V.L., A.S., and V.U., wrote the paper.

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