Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Original Research

Genetic polymorphisms in human telomerase reverse transcriptase (h*TERT*) gene polymorphisms do not associated with breast cancer in patients in a turkish population: hospital-based case-control study

Muhsin Aydin[1], Ahmet Taner Sümbül[2], Günay Camuz Hilaloğullari[3], Süleyman Bayram[4*]

[1] Department of Biology, Faculty of Science and Letters, Adiyaman University, Adiyaman, Turkey
[2] Department of Medical Oncology, Faculty of Medicine, Baskent University, Adana, Turkey
[3] Department of Internal Diseases, State Hospital of Hatay, Hatay, Turkey
[4] Department of Nursing, Adiyaman School of Health, Adiyaman University, Adiyaman, Turkey

Correspondence to: sbayram@adiyaman.edu.tr

Received October 14, 2016; **Accepted** November 03, 2017; **Published** November 05, 2017

Doi: http://dx.doi.org/10.14715/cmb/2018.64.1.3

Abstract: Breast cancer (BC) is encountered most frequently in developed or developing countries. It is the most common cancer in humans following lung cancer, and it is the most common cancer type resulting in mortality in women. Genetic polymorphisms are among the genetic factors that play an important role in the development of the breast cancer. The purpose of this study was to investigate the effect of five functional single nucleotide polymorphisms (SNPs) of h*TERT* (rs2736109 G>A, rs2735940 T>C, rs2853669 A>G, rs2736098 G>A, and rs2736100 T>G) on susceptibility to BC in Turkish population. The genotype frequency of h*TERT* rs2736109 G>A, rs2735940 T>C, rs2853669 A>G, rs2736098 G>A, and rs2736100 T>G polymorphisms were determined by using a polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) and TaqMan methods in 123 subjects with GC and 122 healthy control subjects. The mean age value of the BC patients was 51.58±11.28 (among them 8 subjects ≤35 and 115 subjects >35). In this study, it was found that there was no statistical difference between h*TERT* rs2736109 G>A, rs2735940 T>C, rs2853669 A>G, rs2736098 G>A, and rs2736100 T>G polymorphisms that can be associated with risk of BC.

Key words: Breast cancer; hTERT; Genetic susceptibility; rs2736109 G>A; rs2735940 T>C; rs2853669 A>G; rs2736098 G>A; rs2736100 T>G; Susceptibility.

Introduction

Breast cancer (BC) is the second most common cancer in worldwide and it is the main cause of cancer related deaths among women with an estimated nearly 1.7 million new cases and 522,000 deaths in 2012 (1-3). Breast cancer already is the most common form of cancer in Turkey, with 15,230 diagnosed cases in 2012 (2). This number is predicted to rise to about 25,462 in 2035, according to data from GLOBOCAN 2012 (2). Simply, the number of breast cancer suffering Turkish women will increase by almost 60 percent by 2035. Besides a complicated combination of epidemiological and environmental risk factors, numerous single nucleotide polymorphisms (SNPs) in low-penetrance genes are known to be related with susceptibility, response to treatment and prognosis of BC, proposing a significant contribution of genetic factors to BC (4,5). So, determination and inclusion of functional SNPs to traditional diagnostic methods might be possible to decrease BC-related death by virtue of early diagnosis, patient care, and personalized medicine.

Telomeres are extremely conserved specific ribonucleoprotein complexes and localized at the end of entire linear eukaryotic chromosomes that prevent the chromosomes from degradation, rearrangement, end-to-end fusion, and atypical recombination; thus, telomeres play a crucial role in the preservation of chromosome stability and entirety as well as genomic stability throughout the process of cellular division (6,7). When telomeric tandem nucleotide repeats lengths are progressively shortened to a critical value with every cell cycles, activate the DNA damage checkpoints, drive the cells into the senescence, and finally trigger apoptosis which has been connected with the prevention of the cells against genomic instability and carcinogenesis (6-8). Telomerase recognizes the 3' hydroxyl at the end of the G-strand overhang and adds telomeric tandem nucleotide repeats onto eukaryotic linear chromosome ends (6,8). Telomerase is a ribonucleoprotein that comprise of the telomerase reverse transcriptase (TERT), a telomerase RNA component (TERC) that behaves as a template for DNA synthesis and the protein complex which binds and stabilizes TERC (6). Theoretically, functional genetic variants in h*TERT* gene, which potentially effect telomere length, and activity of telomerase, might play a crucial role in the process of carcinogenesis. The h*TERT* gene, mapped to chromosome 5p15.33, is about 41 kb pairs in size and is composed of 15 introns and 16 exons (9).

As it can be understood from the literature, telomerase plays important roles in carcinogenesis. The main aim of the current study was to investigate whether h*TERT* rs2736109 G>A, rs2735940 T>C, rs2853669 A>G, rs2736098 G>A, and rs2736100 T>G common functional polymorphisms could contribute to any role on susceptibility to BC in Turkish population. To best

of our knowledge, there is no study have been conducted in determining the role of h*TERT* rs2736109 G>A, rs2735940 T>C, rs2853669 A>G, rs2736098 G>A, and rs2736100 T>G polymorphisms in BC susceptibility.

Materials and Methods

Ethics statement

The study was approved by the Human Ethics Committee of the Faculty of Medicine, Mustafa Kemal University (Hatay, Turkey). All the participators ensured their written informed consent to be included in the study concerning the use of their blood specimens for research studies. The study continued in agreement with the statement on the Declaration of Helsinki confirmed by the World Medical Association meeting in Edinburgh.

Study population

This hospital-based case–control study comprised a total of 245 women subjects including 123 BC cases and 122 healthy controls. Clinicopathological features of all subjects are presented in Table 1. Informed consent about the study was taken from all the participants. All participants were over 18 years old and genetically unrelated Turkish and were from the surrounding areas of southern Turkey. Healthy control frequencies were matched to BC cases on age and recruited from volunteers who came to the hospital for their routine checkups. Selection criteria for controls included no evidence of any personal history of cancer or other malignant conditions. All BC cases were newly diagnosed, clinically and histologically confirmed with primary BC and were gathered from the Department of Medical Oncology between October 2013 and November 2014. Classification of BC was carried out according to the seventh edition of the American Joint Committee on Cancer (AJCC) tumor–node–metastasis (TNM) staging system (10).

All clinical and pathological data of patients are taken as follows. Clinicopathological variables of BC patients including age, age at onset, histologic type of cancer, TNM stage, tumor size, lymph node status, nodal status, distant metastasis, histological grade, estrogen receptor (ER) status, progesterone receptor (PR) status, human epidermal growth factor receptor 2 (HER-2/neu) status, lymphovascular invasion, and perineural invasion were collected from the patients' medical records with the help of the oncologist.

DNA extraction

According to the ethical and legal standards, researchers who performed laboratory analysis were blinded to the identification and status of subjects. For this purpose whole blood specimens were handled and made anonymous. Whole blood samples were collected into a test tube containing EDTA from BC patients and healthy controls. None of the BC patients received chemotherapy or radiotherapy prior to whole blood collection. Genomic DNA was isolated from the whole blood specimen of all participants using the AxyPrep Blood Genomic DNAMiniprep KitAP-MN-BLGDNA-250 (Wujiang, Jiangsu, China) according to the manufacturer's directions. The quantity and quality of DNA was identified by the Qubit® Fluorometer (Invitrogen, Carlsbad, CA, USA).

Genotyping of hTERT rs2736109 G>A, rs2735940 T>C, rs2853669 A>G, rs2736098 G>A, and rs2736100 T>G polymorphisms

Genotyping of h*TERT* rs2853669 A>G polymorphism was carried out by using the commercially available TaqMan allelic discrimination assay

Table 1. Clinicopathological features of breast cancer patients.

Variable	N=123 (%)
Age at diagnosis, mean±SD (range 25-77)	51.58±11.28
Age at diagnosis	
≤35	8 (6.50%)
>35	115 (93.50%)
Histologic type of cancer	
Infiltrating ductal carcinoma	100 (81.30%)
Infiltrating lobular carcinoma	13 (10.57%)
Mixed	10 (8.13%)
TNM classification	
I	8 (6.50 %)
II	49 (39.84%)
III	36 (29.27%)
IV	30 (24.39 %)
Tumor Size	
T1	27 (21.95%)
T2	55 (44.71%)
T3	29(23.58%)
T4	12 (9.76%)
Lymph node status	
Negative (-)	26 (21.14%)
Positive (+)	97 (78.86%)
Nodal status	
N0	26 (21.14%)
N1	40 (32.52%)
N2	25 (20.33%)
N3	32 (26.02%)
Distant metastasis	
M0 (Present)	93 (75.61%)
M1 (Absent)	30 (24.39%)
Histological grade	
I (good)	13 (10.57%)
II (intermediate)	63 (51.22%)
III (poor)	47 (38.21%)
Estrogen receptor status	
ER negative (-)	31 (25.20%)
ER positive (+)	92 (74.80%)
Progesterone receptor status	
PR negative (-)	48 (39.02%)
PR positive (+)	75 (60.98%)
HER-2/neu status	
Negative (-)	96 (78.05%)
Positive (+)	27 (21.95%)
Lymphovascular invasion	
Negative (-)	57 (46.34%)
Positive(+)	66 (53.66%)
Perineural invasion	
Negative (-)	81 (65.85%)
Positive (+)	42 (34.15%)

C___8773290_10 (Applied Biosystems Inc., Foster City, CA, USA) according to the manufacturer's directions. Real-time PCR reactions were done in the LightCycler 96 real-time PCR (Roche Diagnostics GmbH, Mannheim, Germany) according to the standard cycling conditions: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, and 60°C for 1 min. Genotype calling for each subject was determined automatically by the LightCycler Genotyping software (Roche Diagnostics GmbH, Mannheim, Germany). PCR was carried out in a total volume of 10 µL containing 5 µL 2× TaqMan®Universal MasterMix II (Applied Biosystems Inc., Foster City, CA, USA), 900 nM of each primer (Applied Biosystems Inc., Foster City, CA, USA), 200 nM of each probe (Applied Biosystems Inc., Foster City, CA, USA), and approximately 10 ng gDNA. The context sequence (VIC/FAM) were (written 5′ → 3′): GTCCCCAGTCCCTCCGCCACGTGGG(A/G)AGCGCGGTCCTGGGCGTCTGTGCCC.

PCR-RFLP analyses were performed to detect the genotypes of the h*TERT* rs2736109 G>A, rs2735940 T>C, rs2736098 G>A, and rs2736100 T>G polymorphisms. The nucleotide sequence of primers, length of amplified DNA fragments, restriction pattern, and restriction enzymes used are presented in Table 2. The 25 µL PCR mixture comprised approximately 125 ng gDNA, with 0.5 µM of both primers, 0.2 mM of each dNTP, 1X PCR buffer, 1.5 mM MgCI2, and 0.5 unit (U) Taq DNA polymerase (Sigma-Aldrich, St. Louis, MO, USA). The following PCR cycling conditions were used: an initial denaturation step of 5min at 95 °C; 30 cycles of 30 s at 94 °C, 30 s at 57 °C (for rs2736109 GNAandrs2735940TNC) or 56 °C (for rs2736100 TNG), and 30 s at 72 °C; a final elongation step of 7 min at 72 °C. As a negative control, PCR mixture without gDNA sample was used to provide a contamination free PCR product. After approval of successful PCR amplification by 1.5% agarose gel electrophoresis, each PCR product was digested overnight with 10 U of restriction endonuclease enzymes (New England Biolabs Inc., Beverly, MA) at 37 °C and electrophoresed on 3% agarose gel containing 0.5 µg/mL ethidium bromide and visualized under UV illumination.

To provide quality control, 15% of the study population were randomly selected without knowledge of the subjects' case-control status and subjected to repeat analysis by different persons; reproducibility was 100%. The success rate of genotyping was 100%, and 123 BC patients and 122 healthy controls were finally included for subsequent statistical analyses.

Statistical analysis

Calculations of power analyses were done prior to starting of the hospital-based case–control study with the Quanto (version 1.1.) software (http://hydra.usc.edu/gxe) (Gauderman and Morrison, 2006). Appropriate sample size for obtaining 80% power was determined by using data of MAFs of h*TERT* rs2736109 G>A, rs2735940 T>C, rs2853669 A>G, rs2736098 G>A, and rs2736100 T>G polymorphisms from the PubMed database of SNPs (dbSNP) (http://www.ncbi.nlm.nih.gov/snp/). Data handling and management as well as statistical analyses were performed by Statistical Package for Social Sciences version 16.0 (SPSS 16.0) software package (SPSS Inc., Chicago, IL, USA). Student's test (for continuous variables) and Pearson's chi-squared ($\chi 2$) test (for categorical variables) were used to compare the differences in the distributions of demographic and clinic characteristics as well as genotype/allele frequencies of h*TERT* rs2736109 G>A, rs2735940 T>C, rs2853669 A>G, rs2736098 G>A, and rs2736100 T>G polymorphisms among BC cases and healthy control subjects. Comparing observed and expected genotypes frequencies of h*TERT* rs2736109 G>A, rs2735940 T>C, rs2853669 A>G, rs2736098 G>A, and rs2736100 T>G polymorphisms in the healthy control group was tested for Hardy–Weinberg equilibrium (HWE) using HWE calculator software (http://www.oege.org/software/hwe-mrcalc.shtml) (Rodriguez et al., 2009). Relations between h*TERT* polymorphisms (rs2736109 G>A, rs2735940 T>C, rs2853669 A>G, rs2736098 G>A, and rs2736100 T>G) and BC risk, haplotype estimation and the corresponding ORs and 95% CIs values, and linkage disequilibrium (LD) were analyzed using online

Table 2. Primer sequences, amplicon size, restriction enzyme and restriction pattern for h*TERT* rs2736109 G>A, rs2735940 T>C, rs2736098 G>A, and rs2736100 T>G polymorphisms.

Polymorphism	Primer sequences	Length (bp)	Restriction enzyme and restriction recognition sites	Restriction pattern (bp)
rs2736109 G>A	F: 5'AAC ATC TGG GTC TGA GGT AGG 3' R: 5'TTA GGA TTA CAG GTC GCT CTT C 3'	471	MboI 5'...↓GATC...3'	A/A: 471 A/G: 471, 284, 187 G/G: 284, 187
rs2735940 T>C	F: 5' ATC TTC TGC TTC CAT TTC TTC TC 3' R: 5' TCG TCT TGT AAA TAC TTA GGA TTC C 3'	235	MspI 5'...C↓CGG...3'	T/T: 235 T/C: 235, 211, 24 C/C: 211, 24
rs2736098 G>A	F: 5′CGT GGT TTC TGT GTG GTG TC 3′ R: 5′ CCT TGT CGC CTG AGG AGT AG 3′	214	PspOMI 5'...G↓GGCCC...3'	G/G: 115, 99 G/A: 214, 115, 99 A/A: 214
rs2736100 T>G	F: 5' CCC CAC AAG CTA AGC ATT AT-3' R: 5'GAA GAA CCA CGC AAA GGA C-3'	152	SfcI 5'...C↓TRYAG...3'	T/T: 152 T/G: 152, 104, 48 G/G: 104, 48

Table 3. Allele and genotype frequencies in the BC case and the healthy control groups as well as association of h*TERT* rs2736109 G>A, rs2735940 T>C, rs2853669 A>G, rs2736098 G>A and rs2736100 T>G polymorphisms with the risk of BC susceptibility according to different models of inheritance.

	Controls n=122 (%)	Breast cancer n=123 (%)	OR (95% CI)	*P*-value[a]	AIC[b]	BIC[c]
rs2736109 (-1600 G>A)						
Allele						
A	101 (41.0%)	99 (40.0%)	1.00 (Reference)			
G	143 (59.0%)	147 (60.0%)	1.05 (0.73-1.50)	0.80		
Codominant						
AA	24 (19.7%)	20 (16.3%)	1.00 (Reference)		344.9	355.4
AG	53 (43.4%)	59 (48.0%)	1.34 (0.66-2.69)	0.42		
GG	45 (36.9%)	44 (35.8%)	1.65 (0.57-2.42)	0.67		
Dominant						
AA	24 (19.7%)	20 (16.3%)	1.00 (Reference)		343.2	350.2
AG+GG	98 (80.3%)	103 (83.7%)	1.26 (0.66-2.43)	0.49		
Recessive						
AA+AG	77 (63.1%)	79 (64.2%)	1.00 (Reference)		343.6	350.6
GG	45 (36.9%)	44 (35.8%)	0.95 (0.57-1.60)	0.86		
Overdominant						
AA+GG	69 (56.6%)	64 (52.0%)	1.00 (Reference)		343.6	350.6
AG	53 (43.4%)	59 (48.0%)	1.20 (0.73-1.99)	0.48		
Log-additive	--	--	1.05 (0.74-1.49)	0.80	343.6	350.6
rs2735940 (-1327 T>C)						
Allele						
C	86 (35.0%)	79 (32.0%)	1.00 (Reference)			
T	158 (65.0%)	167 (68.0%)	1.15 (0.79-1.67)	0.46		
Codominant						
CC	18 (14.8%)	12 (9.8%)	1.00 (Reference)		344.2	345.7
CT	50 (41.0%)	55 (44.7%)	1.65 (0.72-3.76)	0.23		
TT	54 (44.3%)	56 (45.5%)	1.56 (0.68-3.53)	0.29		
Dominant						
CC	18 (14.8%)	12 (9.8%)	1.00 (Reference)		342.2	349.2
CT+TT	104 (85.2%)	111 (90.2%)	1.60 (0.74-3.49)	0.23		
Recessive						
CC+CT	68 (55.7%)	67 (54.5%)	1.00 (Reference)		343.6	350.6
TT	54 (44.3%)	56 (45.5%)	1.05 (0.64-1.74)	0.84		
Overdominant						
CC+TT	72 (59.0%)	68 (55.3%)	1.00 (Reference)		343.3	350.3
CT	50 (41.0%)	55 (44.7%)	1.16 (0.70-1.93)	0.56		
Log-additive	--	--	1.14 (0.79-1.65)	0.47	343.1	350.1
rs2853669 (-245 A>G)						
Allele						
A	157 (64.0%)	167 (68.0%)	1.00 (Reference)			
G	87 (36.0%)	79 (32.0%)	0.85 (0.59-1.24)	0.41		
Codominant						
AA	52 (42.6%)	59 (48.0%)	1.00 (Reference)		344.9	355.9
AG	53 (43.4%)	49 (39.8%)	0.81 (0.48-1.40)	0.46		
GG	17 (13.9%)	15 (12.2%)	0.78 (0.35-1.71)	0.53		
Dominant						
AA	52 (42.6%)	59 (48.0%)	1.00 (Reference)		342.9	349.9
AG+GG	70 (57.4%)	64 (52.0%)	0.81 (0.49-1.33)	0.40		
Recessive						
AA+AG	105 (86.1%)	108 (87.8%)	1.00 (Reference)		343.5	350.5
GG	17 (13.9%)	15 (12.2%)	0.86 (0.41-1.81)	0.69		

Overdominant						
AA+GG	69 (56.6%)	74 (60.2%)	1.00 (Reference)		343.3	350.3
AG	53 (43.4%)	49 (39.8%)	0.86 (0.52-1.43)	0.57		
Log-additive	--	--	0.86 (0.60-1.24)	0.42	343.0	350.0
rs2736098 G>A						
Allele						
G	165 (68.0%)	176 (72.0%)	1.00 (Reference)			
A	79 (32.0%)	70 (28.0%)	0.83 (0.57-1.22)	0.35		
Codominant						
GG	52(42.6%)	63 (51.2%)	1.00 (Reference)		343.4	353.9
GA	61 (50.0%)	50 (40.6%)	0.68 (0.40-1.14)	0.14		
AA	9 (7.4%)	10 (8.1%)	0.92 (0.35-2.43)	0.86		
Dominant						
GG	52 (42.6%)	63 (51.2%)	1.00 (Reference)		341.8	348.8
GA+AA	70 (57.4%)	60 (48.8%)	0.71 (0.43-1.17)	0.18		
Recessive						
GG+GA	113 (92.6%)	113 (91.9%)	1.00 (Reference)		343.6	350.6
AA	9 (7.4%)	10 (8.1%)	1.11 (0.44-2.84)	0.83		
Overdominant						
GG+AA	61 (50.0%)	73 (59.4%)	1.00 (Reference)		341.5	348.5
GA	61 (50.0%)	50 (40.6%)	0.68 (0.41-1.14)	0.14		
Log-additive	--	--	0.82 (0.55-1.22)	0.33	342.7	349.7
rs2736100 T>G						
Allele						
T	89 (36.0%)	90 (37.0%)	1.00 (Reference)			
G	155 (64.0%)	156 (63.0%)	0.99 (0.69-1.44)	0.98		
Codominant						
TT	22 (18.0%)	16 (13.0%)	1.00 (Reference)		342.7	353.2
TG	45 (36.9%)	58 (47.1%)	1.77 (0.84-3.76)	0.14		
GG	55 (45.1%)	49 (39.8%)	1.22 (0.58-2.59)	0.60		
Dominant						
TT	22 (18.0%)	16 (13.0%)	1.00 (Reference)		342.5	349.5
TG+GG	100 (82.0%)	107 (87.0%)	1.47 (0.73-2.96)	0.28		
Recessive						
TT+TG	67 (54.9%)	74 (60.2%)	1.00 (Reference)		342.9	350.0
GG	55 (45.1%)	49 (39.8%)	0.81 (0.49-1.34)	0.41		
Overdominant						
TT+GG	77 (63.1%)	65 (52.9%)	1.00 (Reference)		341.0	348.0
TG	45 (36.9%)	58 (47.1%)	1.53 (0.92-2.54)	0.10		
Log-additive	--	--	1.00 (0.70-1.42)	0.98	343.6	350.6

Table 4. Allele frequencies of hTERT rs2736109 G>A, rs2735940 T>C, rs2853669 A>G, rs2736098 G>A, and rs2736100 T>G polymorphisms according to the 1000GENOMES Data.

Population ID	**h*TERT* polymorphisms**									
	rs2736109		**rs2735940**		**rs2853669**		**rs2736098**		**rs2736100**	
	G allele	A allele	T allele	C allele	A allele	G allele	G allele	A Allele	T allele	G allele
EAS (East Asian)	0.667	0.333	0.478	0.522	0.623	0.377	0.629	0.371	0.585	0.415
EUR (European)	0.606	0.394	0.511	0.489	0.712	0.288	0.765	0.235	0.501	0.499
AFR (African)	0.918	0.082	0.521	0.479	0.924	0.076	0.934	0.066	0.533	0.467
AMR (Ad Mixed American)	0.666	0.334	0.432	0.568	0.761	0.239	0.797	0.203	0.572	0.428
SAS (South Asian)	0.378	0.622	0.671	0.329	0.432	0.567	0.497	0.503	0.395	0.605
Present study (Turkish)	0.586	0.414	0.648	0.352	0.643	0.357	0.676	0.324	0.365	0.635

tool SNPStats: http://bioinfo.iconcologia.net/snpstats/start.htm. To determine the effects of the of h*TERT* polymorphisms (rs2736109 G>A, rs2735940 T>C, rs2853669 A>G, rs2736098 G>A, and rs2736100 T>G polymorphisms) on BC susceptibility, odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by an unconditional logistic regression model adopting co-dominant, dominant, recessive, overdominant, and log-additive models of inheritance. To prefer the inheritance model that best fits the logistic regression data, Akaike's information criterion and Bayesian information criterion were used. A two-sided P-value less than 0.05 were regarded as the statically significant level.

Results

Influence of hTERT rs2736109 G>A, rs2735940 T>C, rs2853669 A>G, rs2736098 G>A, and rs2736100 T>G polymorphisms

A total of 245 Turkish women subjects (122 healthy controls and 123 BC patients) were genotyped in order to determine any possible association between BC and the genotypes of h*TERT* rs2736109 G>A, rs2735940 T>C, rs2853669 A>G, rs2736098 G>A, and rs2736100 T>G polymorphisms. These polymorphisms were detected in the groups of BC cases and healthy control subjects as presented in Table 3. The distribution of h*TERT* polymorphisms genotypes in the healthy control groups were in accordance with Hardy-Weinberg expectation for rs2736109 G>A (P>0.05), rs2735940 T>C (P>0.05), rs2853669 A>G (P>0.05), rs2736098 G>A (P>0.05), and rs2736100 T>G (P>0.05). In this study, the observed frequencies of variant alleles were very similar or close to those reported in East Asian, European, and American ancestry, but different from African and South Asian ancestry (Table 4). The G allele of h*TERT* rs2736109 polymorphism was not associated with BC risk when compared with the A allele of h*TERT* rs2736109 (OR = 1.05, 95% CI 0.73-1.50, *P* = 0.8). Similarly, there was no statistical difference in codominant, dominant, and recessive genetic recessive models for h*TERT* rs2736109 polymorphism in terms of susceptibility to BC (Table 3). The T allele of h*TERT* rs2735940 polymorphism was not associated with BC risk when compared with the C allele of h*TERT* rs2735940 (OR = 1.15, 95% CI 0.79-1.67, *P* = 0.46). Additionally, the codominant, dominant, and recessive genetic recessive models of this polymorphism did not present any significance to be associated with BC risk. Likewise, The G allele of h*TERT* rs2853669 polymorphism was not associated with BC risk when compared with the A allele of h*TERT* rs2853669 (OR = 0.85, 95% CI 0.59-1.24, *P* = 0.41). Moreover, the codominant, dominant, and recessive genetic recessive models of this polymorphism did not present any significance to be associated with BC risk for h*TERT* rs2853669. The G allele of h*TERT* rs2736098 polymorphism was not associated with BC risk when compared with the A allele of h*TERT* rs2736098 (OR = 0.83, 95% CI 0.57-1.22, *P* = 0.35). Also, the codominant, dominant, and recessive genetic recessive models of this polymorphism did not show any significance to be associated with BC. Lastly, the T allele of h*TERT* rs2736100 polymorphism was not associated with BC risk when compared with the G allele of h*TERT* rs2736100 (OR = 0.99, 95% CI 0.69-1.44, *P* = 0.98). This polymorphism also did not present any significant result in the codominant, dominant, and recessive genetic recessive models to be associated with BC.

According to the results presented in Table 3, in all of the genetic inheritance models, none of the h*TERT* rs2736109 G>A, rs2735940 T>C, rs2853669 A>G, rs2736098 G>A, and rs2736100 T>G polymorphisms were showed statistical difference that can be related to BC.

Haplotype Analysis

The total effect of the five h*TERT* polymorphisms (rs2736109 G>A, rs2735940 T>C, rs2853669 A>G, rs2736098 G>A, and rs2736100 T>G) on the BC development was evaluated by haplotype analysis. Haplotype analysis results are presented in Table 5. In total, ten haplotypes were derived from the observed genotypes. Statistically insignificant value of the overall frequencies of haplotypes was observed between BC patients and controls (*P* = 0.07). Grs2736109/Trs2735940/Ars2853669/Grs2736098/Grs2736100 (ht1) was the most common haplotype in BC cases and controls with frequencies of 0.205 and 0.291, respectively. Carriers of Grs2736109/Crs2735940/Ars2853669/Grs2736098/Trs2736100 (ht2) (*P* = 0.02) and carries of Ars2736109/Trs2735940/Grs2853669/Grs2736098/Grs2736100 (ht4) (*P* = 0.04) had an increased risk of BC susceptibility.

The linkage disequilibrium coefficient ($|D'|$) and r^2 among h*TERT* rs2736109 G>A, rs2735940 T>C, rs2853669 A>G, rs2736098 G>A, and rs2736100 T>G polymorphisms was also calculated and presented in Figure 1. rs2736109 G>A and rs2853669 A>G, rs2735940 T>C and rs2853669 A>G, rs2735940 T>C and rs2736098 G>A, rs2735940 T>C and rs2736100 T>G, rs2853669 A>G and rs2736100 T>G were strongly linked to each other with D' = 0.923, r^2 = 0.63; D' = 0.947, r^2 = 0.23; D' = 0.845, r^2 = 0.16; D' = 0.785, r^2 = 0.54; D' = 0.768, r^2 = 0.50; respectively (Figure 1). The remained ones were not in a strong linkage disequilibrium.

Discussion

At the chromosomal termini, erosion of telomeres has always been related with carcinogenesis (10). Chro-

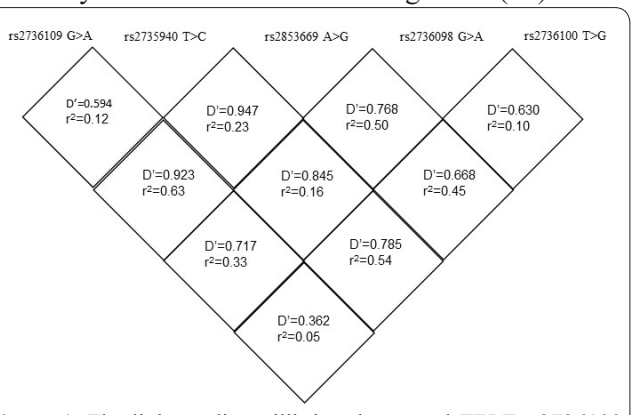

Figure 1. The linkage disequilibrium between h*TERT* rs2736109 G>A, rs2735940 T>C, rs2853669 A>G, rs2736098 G>A, and rs2736100 T>G polymorphisms in Turkish population.

Table 5. Association of h*TERT* haplotypes with the risk of breast cancer.

Haplotype	*hTERT* Polymorphisms					Frequencies		OR (95%CI)	*P*-value
	rs2736109	rs2735940	rs2853669	rs2736098	rs2736100	Control subjects	Breast cancer subjects		
ht1	G	T	A	G	G	0.205	0.291		
ht2	G	C	A	G	T	0.250	0.192	0.53 (0.30-0.92)	**0.02**
ht3	A	T	G	A	G	0.239	0.194	0.63 (0.36-1.11)	0.11
ht4	A	T	G	G	G	0.088	0.049	0.41 (0.18-0.95)	**0.04**
ht5	A	C	A	G	T	0.035	0.070	1.64 (0.57-4.67)	0.36
ht6	G	C	A	G	G	0.036	0.030	0.70 (0.22-2.21)	0.54
ht7	G	T	A	G	T	0.031	0.035	0.74 (0.19-2.87)	0.67
ht8	A	T	G	A	T	0.011	0.047	2.19 (0.51-9.37)	0.29
ht9	G	T	A	A	G	0.034	0.014	0.24 (0.04-1.24)	0.09
ht10	A	T	A	G	G	0.012	0.026	1.55 (0.30-7.90)	0.60
rare	*	*	*	*	*	---	---	0.58 (0.22-1.55)	0.28

Global haplotype association P-value: 0.07.

mosomal stability, cellular proliferation, and the maintenance of telomere DNA length are dependent on the TERT, which is a subunit of the telomerase enzyme (12). In order to understand the tumorigenesis underlying the BC and explore effective prevention and therapeutic approaches, the genetic factors that could be linked to BC susceptibility are needed to be better understood (13,14). In the present study, molecular epidemiological methods were used to find the association between h*TERT* rs2736109 G>A, rs2735940 T>C, rs2853669 A>G, rs2736098 G>A, and rs2736100 T>G polymorphisms on the risk of BC susceptibility and clinicopathologic features of BC through a hospital-based case-control study in a Turkish population.

Many studies have shown that h*TERT* polymorphisms were associated with other cancers' risk. A study, which was done by Peng et al. suggested that h*TERT* rs2736100 polymorphism could increase glioma risk (15)]. Similarly, Zhou et al. also found that h*TERT* rs2736100 polymorphism may contribute to glioma susceptibility (16). Bayram et al. for the first time indicated that h*TERT* rs2736109, rs2735940, and rs2736100 were associated with the risk of gastric cancer susceptibility (17). Liu et al. reported that rs401681 polymorphism is associated with an increased prostate risk, especially among Chinese people (18). Yang and Jiao found that there was a susceptibility of h*TERT* rs2736100 in the development of lung cancer (19). Therefore, this study was conducted in order to determine the association between h*TERT* rs2736109 G>A, rs2735940 T>C, rs2853669 A>G, rs2736098 G>A, and rs2736100 T>G polymorphisms on the risk of BC susceptibility.

According to the meta-analysis that have been done by Li et al., it was suggested that TERT rs2736109, rs2853669, rs2736098, and rs10069690 polymorphisms were associated with increased risk of developing BC. However, in the current study, the obtained results suggested that none of the h*TERT* rs2736109, rs2735940, rs2853669, rs2736098, and rs2736100 polymorphisms can be associated with BC. Another study reported that *HOTAIR* rs920778 polymorphism might play important roles in the genetic susceptibility of BC. Controversially, none of the five tested polymorphisms (rs2736109, rs2735940, rs2853669, rs2736098, and rs2736100) have shown association with BC. Hashemi et al. suggested that the h*TERT* rs2736098 variant influence the risk of BC in an Iranian population in southeast Iran (20). Zhang et al. found that G allele of rs2736100 and G allele of rs2853669 in *TERT* gene, interaction between rs2736100 and smoking, and haplotype containing the rs2736100- G and rs2736109- A alleles were all associated with increased gastric cancer risk (21). Both of these two studies disagree with our findings. However, Shadrina et al. have studied *TERT* polymorphisms rs2853669, rs2736100, and rs7726159 influence on prostate cancer and breast cancer risk in Russian population (22). They have determined that none of the studied polymorphisms have shown an association with the risk of BC. These results are in agreement with the current study.

To conclude, although all cases and controls of this study were collected with very similar inclusion criteria to other studies, some potential factors such as lifestyle and diet which can not be taken into account may have affected the present results. Since there have been many different results in disagreement with the obtained results from this study, were presented in previous studies, further independent studies with larger subject sizes and multiple populations are needed to verify our results.

Acknowledgments
This project was supported by Adiyaman University Research Fund: SYOBAP/2014-0001. Additionally, authors would like to thank all volunteers who participated in this study.

Conflict of interest
Authors declare no conflict of interests.

Author's contribution
Concept: Süleyman Bayram, Muhsin Aydın, Ahmet Taner Sümbül, and Günay Camuz Hilaloğulları; Literature review, data collection or processing: Muhsin Aydın, Süleyman Bayram, Ahmet Taner Sümbül, and Günay Camuz Hilaloğulları; Writing: Muhsin Aydın and Süleyman Bayram.

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2014; 136:359-86.
2. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, et al. GLOBOCAN 2012 v1.0, cancer incidence and mortality worldwide: IARC Cancer Base No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2013. Available from: http://globocan.iarc.fr accessed on 20/10/2017.
3. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2015. CA Cancer J Clin 2015; 65:5–29.
4. Mavaddat N, Antoniou AC, Easton DF, Garcia-Closas M. Genetic susceptibility to breast cancer. Mol Oncol 2010; 4(3):174–91.
5. Yu JC, Ding SL, Chang CH, Kuo SH, Chen ST, Hsu GC, et al. Genetic susceptibility to the development and progression of breast cancer associated with polymorphism of cell cycle and ubiquitin ligase genes. Carcinogenesis 2009; 30(9):1562–70.
6. Sarek G, Marzec P, Margalef P, Boulton SJ. Molecular basis of telomere dysfunction in human genetic diseases. Nat Struct Mol Biol; 22:867–74.
7. Murnane, JP. Telomeres and chromosome instability. DNA Repair (Amst) 2006; 5:1082–92.
8. Kong CM, Lee XW, Wang X. Telomere shortening in human diseases. FEBS J 2013; 280:3180–93.
9. Wick M, Zubov D, Hagen G. Genomic organization and promoter characterization of the gene encoding the human telomerase reverse transcriptase (hTERT). Gene 1999; 232(1):97-106.
10. Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Ann SurgOncol. 2010; 17(6):1471–4.
11. Li ZY, Dong YL, Feng Y, Zhang Z, Cao XZ. Polymorphisms in the telomerase reverse transcriptase promoter are associated with risk of breast cancer: A meta-analysis. J Cancer Res Therapeutics 2016; 12(2):1040-4.
12. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell 2011; 144:646-74.
13. Taylor JG, Choi EH, Foster CB, Chanock SJ. Using genetic variation to study human disease. Trends Mol Med 2001; 7(11):507–12.
14. Ludwig JA, Weinstein JN. Biomarkers in cancer staging, prognosis and treatment selection. Nat Rev Cancer 2005; 5:845–56.
15. Peng Z, Tian D, Chen Q, Zhang S, Liu B, Ji B. Telomerase reverse transcriptase (TERT) rs2736100 polymorphism contributes to increased risk of glioma: Evidence from a meta-analysis. Int J Clin Exp Med 2015; 8:422-30.
16. Zhou P, Wei L, Xia X, Shao N, Qian X, Yang Y. Association between telomerase reverse transcriptase rs2736100 polymorphism and risk of glioma. J Surg Res 2014; 191:156-60.
17. Bayram S, Ülger Y, Sümbül AT, Kaya BY, Genç A, Rencüzoğulları E, Dadaş E. Polymorphisms in human telomerase reverse transcriptase (hTERT) gene and susceptibility to gastric cancer in a Turkish population: Hospital-based case–control study. Gene 2016; 585:84–92.
18. Liu CL, Zang XX, Wang C, Kong YL, Zhang H, Zhang HY. Association between CLPTM1L-TERT rs401681 polymorphism and risk of pancreatic cancer: A meta-analysis. Clin Exp Med 2015; 15(4):477-82.
19. Yang J, Jiao S. Increased lung cancer risk associated with the TERT rs2736100 polymorphism: An updated meta-analysis. Tumour Biol 2014; 35:5763-9.
20. Hashemi M, Amininia S, Ebrahimi M, Hashemi SM, Taheri M, Ghavami S. Association between hTERT polymorphisms and the risk of breast cancer in a sample of Southeast Iranian population. BMC Res Notes 2014; 7:895.
21. Zhang J, Ju H, Gao JR, Jiao XL, Lu Y. Polymorphisms in human telomerase reverse transcriptase (hTERT) gene, gene- gene and gene-smoking interaction with susceptibility to gastric cancer in Chinese Han population. Oncotarget, 2017; 8(12): 20235-43.
22. Shadrina AS, Boyarskikh UA, Oskina NA, Sinkina TV, Lazarev AF, Petrova VD, Filipenko ML. TERT polymorphisms rs2853669 and rs7726159 influence on prostate cancer risk in Russian population. Tumour Biol 2015; 36(2): 841-7.