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Research paper

Polymorphisms in human *telomerase reverse transcriptase* (h*TERT*) gene and susceptibility to gastric cancer in a Turkish population: Hospital-based case–control study

Süleyman Bayram [a,*], Yakup Ülger [b], Ahmet Taner Sümbül [c], Berrin Yalinbaş Kaya [d], Ahmet Genç [e], Eyyüp Rencüzoğullari [f], Erdoğan Dadaş [g]

[a] Adıyaman University, Adıyaman School of Health, Department of Nursing, 02040 Adıyaman, Turkey
[b] Adıyaman University, Education and Research Hospital, Department of Gastroenterology, 02040 Adıyaman, Turkey
[c] Başkent University, Faculty of Medicine, Department of Medical Oncology, 01250 Adana, Turkey
[d] Isparta State Hospital, Department of Gastroenterology, 32100 Isparta, Turkey
[e] Adıyaman Univesity, Vocational School of Health Services, 02040 Adıyaman, Turkey
[f] Adıyaman University, Faculty of Science and Letters, Department of Biology, 02040 Adıyaman, Turkey
[g] Adıyaman Univesity, Faculty of Medicine, Department of Thoracic Surgery, 02040 Adıyaman, Turkey

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ABSTRACT

Erosion of telomeres, tandem nucleotide repeats $(TTAGGG)_n$ that cap the end of eukaryotic chromosomes, has been related with carcinogenesis. The human *telomerase reverse transcriptase* (h*TERT*) gene is encoded the rate-limiting catalytic subunit of the telomerase complexes, which is essential for the protection of telomeric DNA length and chromosomal stability. The purpose of this study was to examine the effect of four functional single nucleotide polymorphisms (SNPs) of h*TERT* (rs2736109 G>A, rs2735940 T>C, rs2853669 A>G and rs2736100 T>G) on susceptibility to gastric cancer (GC) in Turkish population. The genotype frequency of h*TERT* rs2736109 G>A, rs2735940 T>C, rs2853669 A>G and rs2736100 T>G polymorphisms were determined by using a polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) and TaqMan methods in 104 subjects with GC and 209 healthy control subjects. We found that h*TERT* rs2736109 G>A (AA + AG vs. GG OR = 1.68 95% CI = 1.01–2.81, $P = 0.04$), rs2735940 T>C (CC vs. CT + TT: OR = 2.53 95% CI = 1.01–6.13, $P = 0.03$), and rs2736100 T>G (TT vs. TG + GG: OR = 2.27 95% CI = 1.23–4.17, $P = 0.006$) polymorphisms were associated with risk of GC. In the haplotype analysis, *hTERT* Grs2736109/Trs2735940/Ars2853669/Grs2736100 haplotype was also related with an increased risk of GC (OR = 1.75; 95% CI: 1.05–2.93, $P = 0.03$). Because this is the first study regarding the h*TERT* rs2736109 G>A, rs2735940 T>C, rs2853669 A>G and rs2736100 T>G polymorphisms and the risk of GC susceptibility in the literature, further independent studies are needed to verify our results in a larger sample sizes, as well as in patients of different populations.

1. Introduction

Carcinogenesis is a multistep process that arises from the accumulation of multiple genetic anomalies that are the signs of cancers, usually occurring at the chromosome level, resulting in chromosomal instability (Negrini et al., 2010; Sonnenschein and Soto, 2013). Telomeres are extremely conserved specific ribonucleoprotein complexes and localized at the end of entire linear eukaryotic chromosomes that prevent linear 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 (Sarek et al., 2015; Murnane, 2006). 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 (Sarek et al., 2015; Murnane, 2006; Kong et al., 2013).

In humans, telomeres are composed of tandem nucleotide repeats of the $(TTAGGG)_n$ sequence, that are bound by a preservative multiprotein complex also known as "shelterin" or the telosome which has basic roles in the arrangement of telomerase activity (de Lange, 2005; Liu

Abbreviations: ALL, acute lymphoblastic leukemia; BC, breast cancer; CI, confidence interval; GC, gastric cancer; gDNA, genomic DNA; ht, haplotype; h*TERT*, human telomerase reverse transcriptase; HWE, Hardy–Weinberg equilibrium; LC, lung cancer; MAF, minimum allele frequency; OR, odds ratios; PC, prostate cancer; PCR–RFLP, polymerase chain reaction–restriction fragment length polymorphism; SCCHN, squamous cell carcinoma of the head and neck; SNP, single nucleotide polymorphism.

* Corresponding author.
E-mail address: slymnbyrm81@gmail.com (S. Bayram).

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et al., 2004). Telomeric tandem nucleotide repeats progressively shorten throughout every cell division because of ineffective replication of the 3′ end of linear DNA by DNA polymerases (Sarek et al., 2015; Kong et al., 2013). Telomerase recognizes the 3′ hydroxyl at the end of the G-strand overhang and adds telomeric tandem nucleotide repeats onto eukaryotic linear chromosome ends (Sarek et al., 2015; Kong et al., 2013). Telomerase, 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 (Sarek et al., 2015). Human TERT (h*TERT*) expression, encoded by h*TERT* gene, is low in most normal human somatic tissues and is physiologically limited to primary germ line cells, tissue stem cells and activated lymphocytes, leading researchers to take into account hTERT as the rate limiting catalytic subunit of the telomerase complex (Sarek et al., 2015; Zou et al., 2012). Theoretically, functional genetic variants in *hTERT* gene, which potentially effect telomere length, and activity of telomerase, might play a crucial role in the process of carcinogenesis. The h*TERT* lies more than 41 kb and comprise of 16 exons located at 5p15.33 (Wick et al., 1999). The h*TERT* gene is tightly regulated by the transcriptional efficiency of the promoter region (Wick et al., 1999). Transcriptional arrangement of h*TERT* has a very important role in telomerase activity and telomere shortening; for this reason, we especially focused functional genetic variants on the h*TERT* promoter region in this study. A common functional single nucleotide polymorphisms (SNPs) in the promoter region of h*TERT* were detected with a minor allele frequency above 30%, −1600 G>A (rs2736109), −1327 T>C (rs2735940), and −245 A>G (rs2853669) with positions defined by the transcription initiation site as +1 (Horikawa et al., 1999). A few studies have emerged functionality of h*TERT* rs2736109 G>A, rs2735940 T>C, rs2853669 A>G and rs2736100 T>G polymorphisms as mentioned below (Beesley et al., 2011; Matsubara et al., 2006; Hsu et al., 2006; Landi et al., 2009; Codd et al., 2013). First genetic variation, h*TERT* rs2736109 G>A polymorphism locate within the −1600 bp upstream of the promoter region of h*TERT*. To define the functional importance of G → A transition of h*TERT* rs2736109 G>A polymorphism, Beesley et al. (2011), constituted luciferase reporter construct including 3.9 kb of the h*TERT* promoter. They showed that luciferase activity was significantly decreased for the construct carrying A allele of h*TERT* rs2736109 G>A polymorphism in an EOC cell line, a breast adenocarcinoma cell line, as well as in post-selection normal breast epithelial cells (Beesley et al., 2011). For second genetic variation, Matsubara et al. (2006), reported that an h*TERT* rs2735940 T>C polymorphism within the promoter region, a T → C transition −1327 bp upstream of the transcription start site, influences transcriptional efficiency of h*TERT*. In their biochemical studies, they found approximately 25% higher promoter efficiency in the T allele of rs2735940 T>C polymorphism when compared the C allele (Matsubara et al., 2006). In addition to this, individuals carrying homozygous CC genotype of h*TERT* rs2735940 G>A polymorphism displayed shorter telomere length in their peripheral blood leukocytes when compared to the TT and TC genotypes of h*TERT* rs2735940 G>A polymorphism (Matsubara et al., 2006). Third genetic variation, h*TERT* rs2853669 A>G polymorphism is located −245 bp upstream from the "ATG" transcription initiating site of the h*TERT* promoter region, residing within a particular binding motif "GGAA/T" for E-twenty six-2 (Ets-2) transcription factor (Hsu et al., 2006). A transition from A → G of h*TERT* rs2853669 A>G polymorphism results in the disruption of a pre-existing non-canonical Ets-2 core binding site motif adjacent to an E-box, which affect the binding ability of Ets-2 and therefore the transcriptional efficiency and expression level of the h*TERT* gene (Hsu et al., 2006). The G allele of the h*TERT* rs2853669 A>G polymorphism has previously been reported to be associated with low telomerase activity and reduced telomere length in non-small cell lung carcinoma (Hsu et al., 2006). Forth genetic variation, h*TERT* rs2736100 T>G is located in intron 2 of h*TERT* gene and, on the basis of the Evolutionary and Sequence Pattern Extraction through Reduced Representation score, is localized within a putative regulatory region (Landi et al., 2009). Codd et al. (2013), found that people with the TT genotype of h*TERT* rs2736100 T>G had shorter telomeres on average than those with the TG genotype.

In view of the role that telomerase and its regulation seem to play crucial roles in carcinogenesis, it has been proposed that functional genetic polymorphisms in h*TERT* gene can affect individual susceptibility to gastric cancer (GC). In the present study, we sought to investigate whether rs2736109 G>A, rs2735940 T>C, rs2853669 A>G and rs2736100 T>G common functional genetic polymorphisms in the h*TERT* gene could contribute to GC susceptibility in Turkish population. We selected four common genetic polymorphisms (rs2736109 G>A, rs2735940 T>C, rs2853669 A>G and rs2736100 T>G) for the analysis reasoning from a strong evidence for their functionality such as influencing activity of telomerase, telomere length, and genomic stability provided by previous studies (Beesley et al., 2011; Matsubara et al., 2006; Hsu et al., 2006; Landi et al., 2009; Codd et al., 2013). To the best of our knowledge, there is not any study investigating the role of h*TERT* rs2736109 G>A, rs2735940 T>C, rs2853669 A>G and rs2736100 T>G polymorphisms in GC susceptibility. Here, we performed a hospital-based case–control study consisting of 104 GC cases and 209 controls to examine functional SNPs (rs2736109 G>A, rs2735940 T>C, rs2853669 A>G and rs2736100 T>G) in h*TERT* conferring susceptibility to GC in a Turkish population.

2. Material and methods

2.1. Study population

The population of the present study and characteristics of subject were previously defined elsewhere (Bayram et al., 2015). To detect the frequency of four common functional genetic polymorphisms (rs2736109 G>A, rs2735940 T>C, rs2853669 A>G and rs2736100 T>G) of h*TERT* gene in the Turkish population, a 1:2 matched (age and sex) hospital-based case–control study was designed including of 104 subjects with GC and 209 healthy control subjects. This is an ongoing molecular epidemiological study performed in Adıyaman, Turkey, and recruiting study subjects were approved by the Human Ethics Committee of Faculty of Medicine, Adıyaman University (Adıyaman, Turkey). Approval date and number of the human ethics committee as it follows: 06.11.2013 and 2013/11-1.9, respectively. Particularly, all of the subjects were ethnically homogeneous Turkish nationality and residents of the surrounding regions of southern Turkey. Besides, all of the subjects sign their written informed consent form to be included in the study concerning the use of their whole blood specimens for genetic analysis. The study proceeded in agreement with the explanation on the Declaration of Helsinki approved by the World Medical Association conference in Edinburgh. Whole blood specimens were collected from newly diagnosed, clinically and histologically verified with primary GC seen in the Department of Gastroenterology, Medical Oncology and General Surgery between October 2013 and November 2014. The GC cases had not received any treatments such as surgery, radiotherapy, and chemotherapy, when whole blood specimens were drawn from them. During this time, whole blood specimens form healthy controls were gathered from 209 unrelated community residents when they were attending a hospital for their routine general health check-ups.

GC and healthy control subjects were interviewed by physicians using a structured questionnaire to acquire information's such as demographic, clinic, and health characteristics. Detailed subjects characteristics are presented in Table 1.

2.2. DNA isolation

Researchers who performed laboratory analysis were blinded to the identification and status of subjects, because whole blood specimens