Association of Polymorphisms in TCF7L2 Gene with Gastric Cancer Risk: A Preliminary Study in Turkish

TCF7L2 Genindeki Polimorfizmlerin Mide Kanseri Riski ile İlişkisi: Türk Populasyonunda Bir Ön Çalışma

TCF7L2 Geni ve Mide Kanseri Riski / TCF7L2 Gene and Gastric Cancer Risk

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Dudu Erkoç Kaya, Hilal Arıkoğlu, İncilay Çelik Sümen, Ebru Avcı, Özlem Ata, Emine Arslan. Transkripsiyon Faktörü 7 Benzeri-2 gen polimorfizmleri (rs12255372 ve rs7903146) ve Mide Kanseri ile ilişkisi. XIII. Ulusal Tıbbi Biyoloj ve Genetik Kongresi, pp-S, 27-30 Ekim 2013, Kuşadası, İzmir, Türkiye.

Özet

Amaç: Kromozom 10q25.3'te lokalize transkripsiyon faktörü 7-benzeri 2 (TCF7L2) geni birçok kanser tipi ile ilişkisi gösterilmiş, yüksek hareketli grup içeren bir transkripsiyon faktörünü kodlar. Bu faktör, embriyonik gelişimin ve olgun dokularda homeostazinin düzenlenmesinde kilit rol oynayan Wnt/β-katenin sinyal yolağının önemli bir parçasıdır. Wnt/β-katenin sinyal yolağının devamlı aktivasyonunun kanser gelişimine yol açabileceği bilinmektedir. Bu çalışmada, Wnt/β-katenin sinyal yolağının kilit efektörü TCF7L2 genindeki rs7903146C>T ve rs12255372G>T tek nükleotid polimorfizmlerinin mide kanseri ile ilişkisinin ortaya konulması ve ilişkili risk allellerinin belirlenmesi amaçlanmıştır. Gereç ve Yöntem: Çalışmamızda; TCF7L2 genindeki polimorfizmler, 38 mide kanseri hastası ve 48 sağlıklı bireyde, PCR-RFLP tekniği kullanılarak genotiplendirildi. Elde edilen veriler istatistiksel olarak analiz edildi ve tüm değerlendirmelerde p<0.05 anlamlı kabul edildi. Bulgular: rs12255372G>T polimorfizmi additif model altında (OR: 0.366 [95% CI: 0.135-0.989] p= 0.047) hastalıkla ilişkili bulunurken rs7903146C>T ile mide kanseri arasında anlamlı bir ilişki tespit edilmedi (p> 0.05). Tartışma: Çalışmamız, TCF7L2 gen polimorfizmlerinin mide kanseri ile ilişkisinin belirlenmesine yönelik Türk popülasyonunda yapılan ilk araştırmadır ve rs12255372G>T polimorfizminin mide kanseri için bir risk belirteci olabileceğini göstermektedir. Diğer taraftan örnek sayısının artırıldığı daha geniş bir populasyonda yapılan ileriki çalışmalara ihtiyaç vardır.

Anahtar Kelimeler

TCF7L2 Geni; SNP; Mide Kanseri; Türk Populasyonu

Abstract

Aim: The Transcription factor 7-like 2 (TCF7L2) gene, located on chromosome 10q25.3, encodes a transcription factor, which contains a high mobility group box, demonstrated in association with many cancer types. This factor is a critical part of Wnt/β-catenin signaling pathway that plays key roles in regulation of embryonic development and homeostasis in mature tissues. It is known that the constant activation of Wnt/β-catenin signaling pathway can cause cancer development. In this study, it is aimed to reveal the association between rs7903146C>T and rs12255372G>T single nucleotide polymorphisms in TCF7L2 gene, the key effector of Wnt/β-catenin signaling pathway, and gastric cancer and to determine associated risk alleles. Material and Method: In our study, polymorphisms in TCF7L2 gene were genotyped using PCR-PFLP technique in 38 patients with gastric cancer and 48 healthy individuals. The obtained data were statistically analyzed and p<0.05 was accepted significant in all assessments. Results: No significant association was determined between rs7903146C>T and disease (p> 0.05) while 12255372G>T polymorphism was associated with the disease under additive model (OR: 0.366 [95% CI: 0.135-0.989] p=0.047). Discussion: This is the first study to examine the association between TCF7L2 gene and gastric cancer risk in Turkish population and suggests that rs12255372G>T could be a potential indicator for gastric cancer. On the other hand, further studies are required which will be carry out in more increased number of samples in a wider population.

Keywords

TCF7L2 Gene; SNP; Gastric Cancer; Turkish

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Introduction

Gastric cancer (GC) is the fourth most common malign tumor type in the world and the incidence rate between countries and even in different regions of a country shows significant differences [1]. Although numerous biological and epidemiological studies, the mechanisms revealing GC are not yet fully understood but it is known that gastric carcinoma is a very gradual and multifactorial process which genetic and environmental factors are in interaction with each other [2, 3].

Helicobacter pylori infection, smoking, inadequate fruit and vegetable, high meat and salt consumption and lack of food cooling are the major environmental factors [4]. On the other hand, positive family history means high risk for GC [5] and genetic susceptibility accounts for 35% of disease etiology [6]. In recent years, studies have focused on the effects of single nucleotide polymorphisms (SNPs) which thought to cause disease development by altering the functions of a variety of biological pathways in GC. In this context, the association of the SNPs in genes such as NFKB1, PSCA, MUC1, TGFBR1, ERCC4, TOX3 with GC was investigated in various studies conducted in order to reveal the genetic basis of GC [2, 7-9].

The transcription factor 7-like 2 (TCF7L2) gene located on 10q25.3, encodes a high mobility group (HMG) box-containing transcription factor (TCF-4) which is associated with multiple types of cancer [10].This transcription factor is an important part of Wnt/β-catenin signaling pathway that modulates cell proliferation, cell polarity and cell differentiation during embryonic development and also plays a key role on homeostasis in mature tissues [11]. It is known that any mutation which may occur in Wnt/β-catenin signaling pathway causes neonatal defects, osteoporosis, cancer, and other various diseases [11].

Bipartite transcription factor β-catenin/T cell factor (β-cat/TCF), is the major effector of Wnt signaling which is formed by the heterodimerisation of free β-cat with one of the four members of the TCF family [(TCF7 (also known as TCF-1), lymphoid enhancer binding factor (LEF-1), TCF7L1 (also known as TCF-3) and TCF7L2 (also known as TCF-4)] [12]. The active nuclear complex formed by β-catenin and one member of the TCF family; activates the transcription of the Wnt target genes involved in cellular proliferation, evasion of apoptosis, tissue invasion and metastasis [12].

Due to many of these target genes are the proto-oncogenes, the constant activation of Wnt/β-catenin signaling is associated with various cancer types such as colorectal cancer, hepatocellular carcinoma and gastric carcinoma [13-16]. The association of SNPs in various genes with GC was shown in different populations [17]. In the present study, we conducted a case-control association study to evaluate the effect of rs7903146 (C/T) and rs12255372 (G/T) variations in TCF7L2 gene, the key effector of Wnt/β-catenin signaling pathway, on the risk of GC in a Turkish population.

Material and Method

Study subjects

38 patients (12 female, 26 male) diagnosed with GC from Selcuk University, Faculty of Medicine, Department of Oncology and 48 volunteers (29 female, 19 male) with no family history of cancer were included in this study. Informed written consent was obtained from each individual before participation into the study and the Ethical Committee of the Selcuk University, Faculty of Medicine approved the study (decision no:2011/014).

Genomic DNA isolation and genotype analysis

Genomic DNA was extracted from peripheral blood leukocytes using standard proteinase K (Sigma, St Louis, America) and SDS (Sigma, St Louis, America) procedure. The nucleotide sequence of the TCF7L2 gene was obtained from the GenBank™ database. A polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) method was used to genotype rs7903146 (C/T) and rs12255372 (G/T) polymorphisms. PCR amplification was carried out in a 30µl volume containing 50–100 ng genomic DNA, 1X PCR buffer (Fermentase, Vilnius, Litvania), 0.6mM deoxynucleoside triphosphates (Fermentase, Vilnius, Litvania), 0.1 units Taq polymerase (Fermentase, Vilnius, Litvania) and 0.4 µM of each primers (Biomers, Singapur) for rs7903146(C/T) (forward: 5'-GAGAGCTAAGCACTTTTTAGGTA-3' and reverse: 5'-CTGACATTGACTAAGTTACTTGC-3') and for rs12255372 (G/T) (forward: 5'-TGTTAATGGCTTGCAGGTCAG-3' and reverse: 5'-CACCCAAGGTTTGAGGCCTAA-3') polymorphisms. PCR reactions were performed as follows: an initial denaturation at 94 °C for 5 min was followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 65,5°C and 54°C for rs7903146 and rs12255372, respectively for 30 s, elongation at 72 °C for 30 s, and a final extension at 72 °C for 2 min. After amplification , 10µl PCR products were digested with RsaI (Fast Digest, Fermentase, Vilnius, Litvania) at 65˚C for 1 h and with TasI (Fast Digest, Fermentase Vilnius, Litvania) at 37˚C for 1 h for genotyping of rs7903146 (C/T) and rs12255372 (G/T) polymorphisms, respectively. After treatment with restriction enzymes, DNA fragments were visualized on 3% agarose gel electrophoresis and stained with ethidium bromide (Sigma, St Louis, America). Genotyping results were validated by direct sequencing of randomly selected samples for each genotype of all two SNPs.

Statistical analysis

Initial comparison was performed between patient and control groups using t-test. x^2 goodness of fit test was carried out to evaluate Hardy-Weinberg equilibrium (HWE) in patient and control groups. Analyses were carried out using dominant, additive, and recessive models. Dominance was defined in terms of allele 2 (minor allele) effects. In dominant allele 2 models, homozygous individuals for allele 1 were compared with carriers of allele 2. In recessive allele 2 models, homozygous individuals for allele 2 were compared with carriers of allele 1. The odds ratio (OR) and its 95% confidence interval (CI) was obtained by logistic regression method to determine the correlation between the genotypes or alleles of TCF7L2 rs7903146 (C/T) and rs12255372 (G/T) polymorphisms and GC risk by SPSS 18.0 software (SPSS Inc., Chicago, IL). In all analyses, a p value <0.05 was considered statistically significant.

Results

After the incubation with restriction enzymes; the rs7903146 C allele was cut into two fragments of 91 and 22 bp, while the rs7903146 T allele remained uncut (113 bp). For rs12255371

polymorphism; the DNA obtained from the GG homozygote individuals were digested into five fragments (162, 130, 99, 36, and 35bp); from TT homozygote individuals were digested into six fragments (145, 130, 99, 36, 35 and 17bp). When seven bands were visualized, the individuals were genotyped as being GT heterozygotes. According to result of X^2 analyze, both of the SNPs were in Hardy-Weinberg equilibrium ($p<0.05$) except deviation observed in patient group for rs12255372 ($p<0.05$). Genotype distributions of the SNPs were detailed in table 1.

There was no significant association between rs7903146C>T and disease ($p> 0.05$) while 12255372G>T polymorphism was associated with the disease under additive model [OR: 0.366 (95% CI: 0.135-0.989) p=0.047] after odds analyzing carried out under dominant, recessive and additive models.

Table 1. Genotype distributions and results for association analysis between rs7903146 and rs12255372 polymorphisms and gastric cancer

Gene Location	SNP	Genotype n (%)			Odds ratio (95% CI-P)		
					Additive	Dominant	Recessive
Intron 4	rs12255372 G>T	G/G	G/T	T/T	0,366 [0,13-0,99]	0,46 [0,19-1,12]	0,85 [0,22-3,28]
	Patient	17(44,74)	16(42,10)	5(13,15)	p= 0,047	p= 0,089	p=0,819
	Control	31(65,96)	10(21,28)	6(12,76)			
Intron 3	rs7903146 C>T	C/C	C/T	T/T	1,2 [0,31-4,651]	1,6 [0,44-5,62]	0,6 [0,23-1,34]
	Patient	14(36,84)	18(47,37)	6(15,79)	p=0,792	p=0,484	p=0,191
	Control	25(52,08)	18(37,5)	5(10,42)			

Discussion

Gastric cancer (GC), which is the third leading cause of cancer related death in men and the fifth in women worldwide, mostly occurred in countries of Far East such as China and Japan and Eastern European countries [17]. Evidence from several molecular genetics studies indicates that genetic factors of an individual are involved in his/her susceptibility to GC as well as environment and viral/bacterial infections [18]. In a Han Chinese population study, homozygote GG genotype of rs4648068 in NFKB1 gene is associated with increased GC risk [7] while an other Chinese population provided evidence that polymorphism in PSCA gene is involved in susceptibility to GC [8]. Also, rs6478974 and rs10512263 polymorphisms in TGFB1 gene were shown to be associated with GC development under dominant models by Chen et al [9]. Ferrer-Ferrer et al [19] reported an association between HSP70 genotypes and the development of GC in a population in Costa Rica. The SNP rs6983267 is another polymorphism demonstrated to be predisposing the susceptibility of GC in a case-control study whereas Zhang et al [2] showed that rs3803662 in TOX3 gene was significantly associated with survival of GC.

The TCF7L2 gene was firstly reported to be associated with type 2 diabetes (T2D) by Grant et al [20] and has been confirmed to be one of the strongest susceptibility gene of T2D with following studies further. On the other hand, it is hypothesized that TCF7L2 gene may affect cancer independently of diabetes because of its product (TCF4 protein) is involved the Wnt/B-catenin signaling pathway as a transcription factor that induces the expression of target genes such as the cyclin D1 (CCND1) and c-myc oncogenes involved in cellular proliferation, evasion of apoptosis, and also tissue invasion and metastasis [21]. The frameshift mutations in TCF7L2 gene have been found in GC with high microsatellite instability [16]. The association of some SNPs in TCF7L2 gene with various cancer types was shown in recent studies. Of these SNPs, rs7903146 (C>T) polymorphism was reported to be associated with colorectal and lung cancer [22] prostate cancer and increased breast cancer risk [23]. Neverthless, Connor et al [10] determined three polimorphisms rs3750805, rs7900150 and rs1225404 as well as rs7903146 in TCF7L2 to be associated with breast cancer. Rs12255372 (G>T) was shown to be associated with familial breast cancer risk [23] while it was shown to increase prostate cancer aggressiveness in another study [24]. Results of a meta-analysis indicated that there was a significant association between TCF7L2 rs7903146 (C>T) polymorphism and the risk of breast, prostate and colon cancer rather than colorectal, lung and ovarian cancer [21]. Although numerous studies have shown different genes and variants as genetic risk factors for gastric cancer, revealing the exact molecular mechanism of GC is still a challenge. The strong candidate TCF7L2 gene, as a transcription factor, may enhance the canceration of gastric epithelial cells by changing the expressions of a variety of genes involved in cell cycle such as c-myc oncogenes. In our country, although it is the second most common type of cancer subsequently breast cancer in women and lung cancer in men [25], there is no study on the genetic basis of GC. Up to now, the associations between polymorphisms in various genes and GC have been investigated in several studies worldwide, but the association between TCF7L2 gene polymorphisms and the GC risk was not investigated in any study to the best of our knowledge. We investigated the risk associated with rs12255372(G>T) and rs7903146(C>T) SNPs in TCF7L2 gene for the first time in a Turkish population with GC.

In our study we did not detect a significant association between rs7903146C>T and GC ($p> 0.05$) but rs12255372G>T polymorphism was significantly associated with the disease risk. Our findings suggest that rs12255372 (T) variant may increase the susceptibility of GC, thus could be a potential indicator for GC risk. Lack of association between rs7903146 and GC could be due to our relatively small sample size. The other limitations of the present study is; not determining the effect of gene-gene/gene environment interaction and of analyzing stratified of tumor size, histological type, dept of invasion, drinking status, information of tumor site, etc. However, it is the first study which investigates the association between TCF7L2 gene polymorphisms and GC which presents the initial data obtained from Turkish population about whether TCF7L2 gene polymorphisms are associated with GC.

Further studies are required to confirm our findings and the role of these variants in GC susceptibility in larger sample sizes among ethnically and geographically different populations.

Competing interests

The authors declare that they have no competing interests.

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