Research Article

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The influence of *CASP8* D302H gene variant in colorectal cancer risk and prognosis

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Abstract

Objectives: Apoptosis is defined as programmed cell death, which regulates cellular functions and various physiological responses. Several studies reported that *Caspase* genes play important roles in the apoptosis and inflammation process. *Caspase-8 (CASP8)* is a member of the cysteine protease family and a key regulator gene in the induction of apoptosis. In present study, we aimed to investigate the possible associations between the *CASP8;* D302H (G>C) gene polymorphism and colorectal cancer risk and prognosis.

Methods: The *CASP8*; D302H genotypes were determined in 75 colorectal cancer patients and 122 healthy controls. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism method (PCR-RFLP) was used to detect the *CASP8;* D302H gene variation in the study group.

Results: We found that individuals carrying the GC genotype of *CASP8*; D302H gene variation had significantly lower colorectal cancer risk compared with those carrying CC and GG genotypes (OR=0.539; p=0.045). In addition, we analyzed the clinicopathological characteristics of patients and noticed a significant correlation between the C allele frequency and moderately differentiated tumor parameter (p<0.05).

Conclusions: The *CASP8*; D302H gene polymorphism GC genotype might be associated with a reduced risk of colorectal cancer but further studies in a larger population are needed most effective evaluation of the *CASP8*; D302H gene variation in colorectal cancer development.

Keywords: apoptosis; caspase-8; colorectal cancer; gene variation; prognosis.

Introduction

Colorectal cancer (CRC) is a common type of malignancy with an increasing incidence tendency, in terms of both morbidity and mortality [1]. The pathogenesis of CRC is induced by multiple variables such as genetic, epigenetic factors, and alterations in protein expression also it is known that influenced by host immune reactions [2, 3]. Advanced scientific improvements have led to the investigation of novel biomarkers to clarify the possible risks of diseases and enhance our understanding of health problems and predict therapeutic approaches [2, 3].

Apoptosis is programmed cell death also a crucial mechanism to maintain homeostatic balance between cell growth, division, and death [4, 5]. Dysregulation of this death mechanism, which consists of the receptors, ligands, adaptors, and caspases, induces carcinogenesis by activating cell proliferation and genetic variations also promotes resistance to therapy [5, 6].

Caspases (cysteine aspartyl proteases) are key regulators of apoptosis, which hydrolyze aspartic acid peptide bonds within crucial intracellular molecules to induce cell death [7–10]. Several reports have concluded that the caspases influence immunological functions, cell proliferation, and migration [11]. According to studies, apoptosis is regulated by two basic pathways, which are defined as the internal pathway (mitochondria-related) and the external (extrinsic) pathway. The extrinsic pathway includes proteins, which were identified as tumor necrosis factor (TNF) family (TRAIL-R1, TRAIL-R2), membranous receptor Fas/CD95, and death receptors (DRs) [11]. *Caspase-8* (CASP-8) initiates the extrinsic apoptotic pathway by activating tumor necrosis factor receptor 1 (TNFR)1, cell surface death receptor (FAS), and (DRs) [12, 13].

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Studies have reported that the binding of receptor-ligand complex, catalytically induces *Caspase-8* through homo-oligomerization/dimerization, subsequently, the activated *Caspase-8* transfers the death signal to mainly important executor caspases such as *Caspase-3,* which then cleave several cellular proteins to complete the apoptosis-inducing process [14].

The first genome-wide association studies (GWAS) enable to the identification of many genomic polymorphisms and mutations, which are potentially associated with cancer risk and development [15]. The human *CASP8* gene is located on human chromosome 2q33–34 and it has at least 11 exons spanning ~30 kb, which contains highly polymorphic regions [16]. *CASP8;* D302H (aspartate to histidine, G>C; rs1045485) gene polymorphism has been analyzed in many types of cancers [17–21]. Although in several studies researchers concluded that the substitution of an amino acid (the aspartate to histidine) at the D302H region theoretically might influence the auto processing of *Caspase-8* and molecular interactions, the mechanism is yet to be confirmed by several studies, which are conducted in different populations [21].

To the best of our knowledge, there was no study that mainly focused on *CASP8*; D302H gene variation and colorectal cancer risk in the Turkish population, so our purpose in the present study was to investigate the possible correlations between the *CASP8;* D302H gene polymorphism and colorectal cancer risk.

Materials and methods

Sample groups

In the present study, 75 colorectal cancer patients and 122 healthy individuals were analyzed. The whole cases were selected from Istanbul Education and Research Hospital. This case-control study was approved by the Istanbul University Faculty of Medicine Ethical Committee and the research protocol was consistent with the World Medical Association Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects). The Power and Sample Size Calculation Program software were used to evaluate effective sample size and the data size was consistent. The peripheral blood samples were taken into EDTA containing tubes from individuals who have given informed consent. The patient samples were obtained before chemotherapeutic or radiation therapy. The verification analysis of histological grade was determined through the assessment of differentiation.

Extraction of DNA

A common and reliable technique, salting-out was used for extracting genomic DNA from blood samples [22]. Briefly, 5–10 mL of fresh blood samples were transferred to 50 mL falcon tubes and the cell lysis buffer was added and incubated for 10 min. After centrifugation, the supernatant was discarded. These steps were done twice. White blood cell lysis, 10 % SDS, and proteinase K (20 mg/mL) were included in the pellet and incubated in a water bath at 56 °C overnight. Then, 9.5 M ammonium acetate was added, and the samples were shaken a few times and centrifuged. The supernatant was taken into a clean tube and 99 % ethanol was added. After this step, visible DNA threads were taken with a clean tip and stored at appropriate pH and temperature until analysis.

SNP genotyping

The *Caspase-8*; D302H gene variation was detected by PCR–RFLP (polymerase chain reaction–restriction fragment length polymorphism) method. We used to following primer sequences: 5′-CATTTTGAGATCAAGCCCCG-3′ (forward) and 5′-CCCTTGTCTCCATG GGAGAGGA-3′ (reverse) for the amplification. The PCR reaction started with the initial DNA denaturation step at 95 °C for 5 min, followed by 35 cycles at (95 °C for 45 s, 60 °C for 45 s, 72 °C for 45 s). The final extension step was at 72 °C for 4 min. *Caspase-8;* D302H polymorphism, 132-bp PCR products were digested by *BstUI* (MBI Fermantas) at 37 °C for 3 h. After incubation, all of the digested products were visualized in 3 % agarose gel containing ethidium bromide, and band sizes were determined with markers of 50 bp. The GG genotype generated two fragments of 112, 20 bp, and the CC genotype individuals were identified by the presence of 132 bp in the agarose gel [21].

Statistical analysis

The data were evaluated with SPSS version 21 for Windows (SPSS Inc, Chicago, IL, USA). Numerical results were defined as mean, standard deviation, and percentage. The determination of *CASP8;* D302H genotype frequencies in the patients and the healthy group were performed by using the chi-square test. Odds ratios (ORs) with 95 % confidence intervals (CIs) were calculated for risk estimation. A p-value less than 0.05 was considered significant. The distribution of allele and genotype frequencies was determined according to Hardy-Weinberg equilibrium (HWE) in the study population ($p>0.05$).

Results

The characteristics of colorectal cancer patients were given in Table 1. We didn't find statistically significant differences in terms of age, family history, or smoking status parameters among the study groups in the present study. The mean age of participants was 59.31 ± 13.1 years in cases and 53.11 ± 10.39 in healthy individuals respectively. The distribution of *CASP8*; D302H polymorphism genotype and allele frequencies in study groups were given in Table 2. The *CASP8*; D302H (GG, GC and CC) genotypes frequencies were observed as (54.1 , 45.1, 0.8 %) in controls and (66.7 , 30.7, 2.7 %) in patients respectively. In present study, we found that the individuals with GC (heterozygote) genotype had significantly lower colorectal cancer risk compared

with those carrying CC and GG genotypes of *CASP8*; D302H polymorphism (OR=0.539; p=0.045) (Table 3).

On the other hand, we detected the CC genotype in only two patients and this rare genotype was observed in only one healthy individual. For this reason, carrying the CC + GC genotypes (C recessive allele) of *CASP8*; D302H polymorphism were compared with possessing the GG (homozygote of dominant allele) genotype to evaluate whether a statistical link between cases and controls in terms of allele and genotype distributions, but we did not observe any significant differences. In addition, we investigated the potential correlations between the *CASP8;* D302H, allele frequencies, and the prognostic features of cases. The data was shown in Table 4. We observed that possessing the C allele of *CASP8*; D302H polymorphism was associated with moderately differentiated tumor parameter (OR=1.17; p<0.05) but there was not any statistical significance between the *CASP8;* D302H gene variation and other histopathologic characteristics of the patients.

Discussion

Colorectal cancer (CRC) incidence has increased in recent decades. The progression of colorectal cancer malignancy is a multistep process, which consists of alterations in molecular mechanisms, instability between cell growth and apoptosis, and long-term accumulation of genetic aberrations [23, 24].

Table 1: Characteristics of patients with colorectal carcinoma (%).

	Patients, %
T stage	
T1 + T2	26.9
T3 + T4	73.1
Lymph node status	
N0	42.3
N1 + N2 + N3	57.7
Differentiation	
Well	28.6
Moderate poor	71.4
Distant metastasis	
Absence	81.5
Presence	18.5
Perineural invasion	
Absence	53.8
Presence	46.2
Angiolymphatic invasion	
Absence	61.6
Presence	38.4

Table 2: Genotype and allele frequencies of cases and controls.

Genotype and allele	Patients n=75, %	Controls n=122, %	p-Value
GG	50 (66.7)	66 (54.1)	p>0.05
GC	23 (30.7)	55 (45.1)	
CC	2 (2.7)	1 (0.8)	
G Allele	123 (82)	187 (76.6)	0.207
C Allele	27 (18)	57 (23.3)	

n, number of individuals, chi-square ($\chi 2$) test was used between the groups. p-values less than 0.05 denote statistical significance.

Table 3: The risk of colorectal cancer associated with *CASP8* D302H.

	Patients, n (%)	Controls, n (%)	OR	(95 % CI)	p-Value
GG + GC	73 (97.3)	121 (99.2)	0.302	0.027–3.385	0.559
CC	2 (2.7)	1 (0.8)			
CC + GC	25 (33.3)	56 (45.9)	0.589	0.324–1.071	0.082
GG	50 (66.7)	66 (54.1)			
GG + CC	52 (69.3)	67 (54.9)	0.539	0.294–0.988	**0.045**[a]
GC	23 (30.7)	55 (45.1)			

OR, odds ratio; CI, confidence interval. [a]p-Value <0.05 denoted statistical significance.

Table 4: Distribution of the *CASP8* D302H genotypes with different histopathological parameters.

Tumor characteristics	GG + GC, %	CC, %	p-Value	CC + GC, %	GG, %	p-Value
T Stage						
T1 + T2	29.2	n/a	p>0.05	22.2	29.4	p>0.05
T3 + T4	70.8	100		77.8	70.6	
Lymph node status						
N0	45.8	n/a	p>0.05	22.2	52.9	p>0.05
N1 + N2 + N3	54.2	100		77.8	47.1	
Differentiation						
Well	30.8	n/a	p>0.05	n/a	42.1	**0.029**[a]
Moderate poor	69.2	100		100	57.9	
Distant metastasis						
Absence	80	100	p>0.05	80.0	82.4	p>0.05
Presence	20	n/a		20.0	17.6	
Perineural invasion						
Absence	45.8	50	p>0.05	50.0	43.8	p>0.05
Presence	54.2	50		50.0	56.3	
Angiolymphatic invasion						
Absence	62.5	50	p>0.05	60.0	62.5	p>0.05
Presence	37.5	50		40.0	37.5	

n/a, not available. [a]p-Value <0.05 denoted statistical significance.

Caspase genes, promote cellular maturation and reconstruction by initiating the apoptosis of old cells or that could not able to perform their functions, so they maintain the balance between cell life and death [25, 26]. The

Caspase-8 (CASP8) gene has critical roles in the regulation of apoptosis and inflammatory process [27]. Although several studies reported that the loss of *Caspase-8* expression is not detected in most epithelial-derived cancers such as colon carcinoma, gastric cancer, and breast cancer, many reports concluded, that the *CASP8* mutations have been observed in 5 % of invasive colorectal carcinomas (but not adenomas) and these genetic variations might lead to the loss of *CASP8* apoptotic function. Moreover, they affect the pathogenesis of colorectal carcinomas [28, 29].

According to the importance of *Caspase genes* in carcinogenesis, we evaluated the *CASP8*; D302H gene variation in colorectal cancer risk and also aimed to reveal if there were any associations with the clinicopathological characteristic of the patients. We observed that individuals carrying the GC genotype of *CASP8;* D302H polymorphism had significantly reduced colorectal cancer risk compared with those carrying CC and GG genotypes (p=0.045). When we stratified histopathological features of the patients and analyzed them according to genotype and allele frequencies, we found that the possessing of the C allele was associated with moderate tumor differentiation parameter (OR=1.17, 95 % CI=1.177–2.535; p<0.05).

The *CASP8*; D302H gene polymorphism has been analyzed in different types of cancers [17–21], but in the literature, there was a limited study that directly focused on the *CASP8*; D302H gene variation and colorectal cancer risk. In a study, which was conducted to evaluate the *CASP8*; D302H (rs1045485), *CASP8*; (rs3834129) gene variants and risk of CRC in the UK population, Pittman et al. concluded that there was no association between both variants and risk of CRC progression in their data analysis [30]. In another study, Hashemi et al. designed an investigation to assess the possible roles of *CASP8;* rs1045485 G>C, rs3834129, rs3769818 G>A, rs6723097 A>C, rs3769821 T>C, rs13113T>A, rs3769825 G>A, rs2293554 A>C, and rs10931936 C>T polymorphisms in susceptibility of cancers. They reported that the *CASP8*; (rs3834129) polymorphism was associated with the reduced risk of gastrointestinal, digestive tract, colorectal, breast, and lung cancers and concluded the *CASP8;* D302H polymorphism was related to the decreased risk of overall cancer [31]. Vahednia et al. conducted a study in the Iranian population and found that the CC and GC genotypes of *CASP8;* D302H polymorphism were significantly increased in healthy controls compared to breast cancer patients. As a result, they concluded the C allele of *CASP8*; D302H has a protective impact on breast cancer risk in the Iranian population [21].

In a meta-analysis, which was conducted by Zhang et al., they reported that the *CASP8;* D302H gene variation was significantly associated with decreased breast cancer risk in the population of the UK, Germany, and Poland but they did not observe a significant relationship in Finland or USA populations [32].

Bethke et al. conducted a study to test the possible effect of *CASP8*; D302H polymorphism in the risk of glioma. They concluded that carrying the rare allele of D302H variation was associated with an increased risk of glioma [33]. Similarly, they designed a study to analyze the *CASP8*; D302H polymorphism and susceptibility of meningioma through investigation of five independent series of case-control studies. They did not find a statistically significant correlation between the carrying *CASP8*; 302H variant and the risk of meningioma [34]. On the other hand, Lubahn et al. analyzed three independent series of aggressive prostate cancer cases and control studies. They observed that the H allele of *CASP8;* D302H polymorphism was related to decreased risk of aggressive prostate cancer [35].

In conclusion, this is the first study, which was investigated the association between the *CASP8*; D302H gene polymorphism and colorectal cancer risk in the Turkish population. Although the GC genotype of *CASP8*; D302H gene variation was observed as significantly associated with reduced risk of colorectal cancer, our study has a limitation that needs to be mentioned. This was a preliminary report and the size number of the study group was relatively small, so larger population data might provide to determine underlying mechanisms of *CASP8*; D302H polymorphism in the colorectal cancer risk and prognosis.

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