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Caspase-9 rs1052576 Polymorphism is not Associated with Glioblastoma in Turkish Patients

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ABSTRACT

Objective: As an aggressive type of brain cancer, glioblastoma remains obscure with its short survival time and unclear molecular architecture. Single nucleotide polymorphisms (SNPs), such as those located in the regions of the caspase-9 gene (*CASP9*), have been reported to be associated with genetic susceptibility to glioblastoma. There is no exact result on the effect of *CASP9* SNP rs1052576 on glioblastoma and its biomarker candidacy for the Turkish population. Investigating the polymorphism of the exon 5 (+32 G/A) region of the *CASP9* in glioblastoma patients in the Turkish population was the aim of this study.

Materials and Methods: Real-time polymerase chain reaction (RT-PCR) method on blood samples of glioblastoma patients (n=33) and healthy controls (n=35) were used to analyze *CASP9* SNP rs1052576. Statistical data were obtained using SPSS v.23 software.

Results: For *CASP9* rs1052576, no evidence was found of its role in glioblastoma (p=0.594).

Conclusion: This study was designed to determine the association between glioblastoma and *CASP9* SNP rs1052576 within the Turkish population. Our results indicated that *CASP9* SNP rs1052576 is not related to glioblastoma in Turkish patients. To clarify these results, further studies with a larger sample size are needed.

Keywords: Caspase-9, glioblastoma, polymorphism, rs1052576

INTRODUCTION

As an aggressive type of adult primary brain tumor, glioblastoma is known for its poor survival rate (average survival of only 8 months) (1). It constitutes about 14.0 % of all primary (2) and at least 60 % of all brain tumors in adults (3). Necrosis, hypercellularity, microvascular proliferation, and nuclear atypia are some histological features of glioblastoma (4). Diverse genetic factors and epigenetic alterations are responsible for the development and progression of glioblastoma (5, 6). Decreased apoptosis, one of the reasons for the aggressive characteristics of the disease, and intrinsic deregulation in apoptotic cell death are seen in this multifactorial disease (7).

In human cancers such as glioblastoma, evasion of apoptosis is accepted as a hallmark (8). Cell proliferation and cell death homeostasis is maintained by apoptotic mechanisms (7). There are two identified apoptotic pathways, namely: Extrinsic (triggered through membrane death receptors) and intrinsic (activated by cellular stresses) also called the mitochondrial pathway (9). A defect in apoptosis underpins the tumorigenesis and malignant progression of glioblastoma (10). Because of

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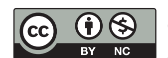

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their crucial roles in apoptosis, caspases are at the center of studies on carcinogenesis. Caspase-9, as a member of this conserved cysteine-aspartic proteases family, takes place in the regulation of intrinsic apoptosis as an initiator, and it is indispensable for efficient apoptosis. Activation of caspase-9 results in the induction of apoptotic cell death by cleavage of effector caspases. Cellular damage can also cause a response by triggering intrinsic apoptosis and tumor growth can be suppressed by caspase-9. However, as a consequence of the evasion of apoptosis, caspase-9 can be inhibited by some tumors. The apoptotic potential of caspase-9 is regulated by processes such as interference with its binding to the apoptosome. Further, some inactivating mutations and certain polymorphisms can affect the abundance or activity of caspases (11). Polymorphisms in genes regulating apoptosis can determine the efficiency of apoptosis (8). Not only at the gene level, but also at the single nucleotide polymorphism (SNP) level, alterations of caspase-9 gene (*CASP9*) have been associated with human cancers (11). The human *CASP9*, which encodes an apoptosis-related cysteine protease, caspase-9, is located on chromosome 1 region p36.2 (12). In databases, a few candidate *CASP9* SNPs have been listed (13). Some of these SNPs as potential biomarkers are found in the promoter (e.g. rs4645978, rs4645981) or exon sequence of *CASP9* (e.g. rs1052576).

Susceptibility to various cancers, including lung cancer, colorectal cancer, liver cancer, and prostate cancer, can occur through *CASP9* polymorphisms, such as the ones aforementioned, influencing caspase-9 expression (14). Among the *CASP9* polymorphisms, there are only a few studies that have examined whether the *CASP9* exon 5 (+32 G/A) SNP rs1052576 is a predisposing factor to human diseases or not (15). As a consequence of this exonic polymorphism at codon 221, glutamine amino acid is converted to arginine (Q221R). As a result of this substitution, a conformational change occurs in the caspase-9 molecule, and the apoptotic mechanism is influenced through modification of the binding of caspase-9 to Apaf-1 (16). Based on all this information, the role of *CASP9* SNP rs1052576 in glioblastoma was investigated in this study, which was performed in the Turkish population.

MATERIALS AND METHODS

Study Population and Clinical Procedures

In this study, 68 individuals were analyzed (including 35 controls and 33 glioblastoma cases). All participants were selected and recruited from Yeditepe University Neurosurgery Department (Istanbul, Turkiye) after detailed clinical examinations and whole blood samples were taken. This study was performed per tenets of the Declaration of Helsinki. Ethical approval was obtained for this study from the Yeditepe University Medical Faculty Ethics Committee (Date: 24.10.2018 and No: 916). Data about the clinical and demographic characteristics of the study groups were registered before the experimental phase of the study. Also, all individuals signed an informed consent form.

CASP9 SNP rs1052576 Genotyping

Following ethical procedures, EDTA tubes were used to collect blood samples from all individuals. An iPrep Purification device (Invitrogen, Life Technologies, Carlsbad, California, USA), 350 µL of peripheral blood, and an Invitrogen iPrep PureLink gDNA blood isolation kit (Invitrogen, Life Technologies, Carlsbad, California, USA) were used in the DNA extraction procedure. NanoDrop 2000 (Thermo Scientific, Waltham, Massachusetts, USA) was chosen to measure the genomic DNA purity and its concentration. The dilution concentration of the DNA samples was 100 ng/µL. Genotyping for *CASP9* SNP rs1052576 was performed by an Applied Biosystems 7500 Fast Real-Time polymerase chain reaction (RT-PCR) device. The components of the PCR master-mix were 1 µl DNA template, 0.25 µL TaqMan Genotyping Assay, 5 µL TaqMan Genotyping Master Mix (TaqMan Reagents, Applied Biosystems, Foster City, CA, USA), and 3.75 µL DNase free water. The holding stage at 95˚C for 10 min, the denaturation stage at 92˚C for 15 sec (40 cycles), and the binding/elongation stage at 60˚C for 1 min were the conditions of the PCR using sequence-specific primers (Table 1).

Statistical Analyses

Obtained data were analyzed using SPSS 23 program (SPSS Inc., Chicago, IL, USA). Student's t-test was conducted to compare the significance of the differences between groups. Chi-square and Fisher's exact tests were used to determine the difference in the existence of target polymorphism in the patient and control groups. Statistical significance was assessed by at least $p<0.05$.

RESULTS

Table 2 shows the demographic characteristics of the study participants. Of the 68 individuals, 33 were patients (72.30% male, 27.70% female) with a mean age of 48.69±18.34 years and 35 individuals were healthy controls (62.90% male, 37.10% female) with a mean age of 42.75±11.70 years . The mean age ($p=0.108$) and gender ($p=0.386$) of the study populations were not significantly different.

Table 1. The polymorphism and experimental details

Gene, chromosome location, base change, SNP	Genotyping method	Primers
CASP9, 1p36.21 Ex5 +32G > A, rs1052576	PCR	5'-GGCTTTGCTGGAGCTGGCCC-3' (sense) 5'-AGTACCCAATGCCTGCCCAGGG-3' (antisense)

Table 2. Demographic data of the study subjects

Characteristic	Patient (n=33)	Control (n=35)	p-value
Gender (%)	Male / Female 72.3 / 27.7 (n=24) / (n=9)	Male / Female 62.9 / 37.1 (n=22) / (n=13)	0.386
Age (year) (mean ± SD)	48.69 ± 18.34	42.75 ± 11.70	0.108

n: Number of the sample, SD: Standard deviation

Table 3. Distribution of *CASP9* SNP rs1052576 genotypes and alleles in cases and controls

Polymorphism rs1052576		Case (n=33) n (%)	Control (n=35) n (%)	p-value	Odds Ratio	95%-CI%
Exon 5 +32 Genotypes						
	GG	11 (33.3)	16 (45.7)	0.493	1.3714	0.5559 - 3.3835
	GA	18 (54.5)	14 (40.0)	0.471	0.7333	0.3150 - 1.7072
	AA	4 (12.2)	5 (14.3)	0.817	1.1786	0.2912 – 4.7706
Alleles	G	40 (60.6)	46 (65.71)	0.803	1.0843	0.5735 – 2.0501
	A	26 (39.4)	24 (34.29)	0.709	0.8703	0.4191 – 1.8074

n= Number of the sample, CI: Confidence interval

Table 3 summarizes the information on genotype and allele frequencies for *CASP9* SNP rs1052576 in the study groups. According to the information in Table 3, it is seen that there is no statistical significance in the *CASP9* SNP rs1052576 genotype frequencies between groups (p>0.05). Out of 33 glioblastoma patients, the frequencies of the GG:GA:AA genotypes were 11:18:4. Out of 35 healthy individuals, the frequencies of GG:GA:AA genotypes were 16:14:5. While the frequency of *CASP9* rs1052576 G-allele was 60.60% in those with glioblastoma, it was 65.71% in the control group. In 39.40% of patients and 34.29% of healthy individuals, the mutant A allele was present. The *CASP9* ancestral G allele and mutant A allele were not associated with glioblastoma (p=0.803 and p=0.709, respectively) (Table 3).

DISCUSSION

It is known how important apoptotic mechanisms are in diseases with genetically complex architecture such as glioblastoma. Examining molecules such as caspase-9, which has a key role in the regulation of intrinsic pathways, is important in elucidating the molecular mechanism of the disease. Though SNPs are not considered to be the only causative factors of glioblastoma, they are important genetic factors in disease susceptibility because of their impact on gene regulation and its products. Hence, this study was initiated to analyze the place of *CASP9* SNP rs1052576 in glioblastoma.

This target SNP at codon 221 in exon 5 of the *CASP9* causes the substitution of glutamine by arginine (Q221R) (15). Some studies have suggested that this Q221R variant might influence carcinogenesis due to conformational changes in caspase-9. The conformational changes lead to alterations in the binding affinity of caspase-9 for Apaf-1 (14, 16). Despite previous studies reporting a significant relationship of this SNP with several human diseases (15), in our Turkish cohort of glioblastoma patients, a genetic link between the *CASP9* SNP rs1052576 and glioblastoma has not been detected.

In the literature, there are studies examining *CASP9* polymorphisms and their impact on cancer development and progression in different cancer types, including multiple myeloma (17), and non-Hodgkin's lymphoma (18). These studies showed that *CASP9* SNP rs1052576 decreased susceptibility to the diseases. Furthermore, three different comprehensive meta-analyses have shown that the A allele of rs1052576 is protective against cancer (16, 19, 20). The impact of *CASP9* polymorphisms (rs4645978, rs1052576, and rs4645981) on cancer susceptibility has been shown via investigations performed by Xu et al. They have reported that

carriers of the A allele of rs1052576 in the Asian subgroup have less risk for cancer (14). Mutations in the *CASP9* have been analyzed by Yan et al. in Chinese, American, Russian and Caucasian populations. According to the results of this study, while the A allele is protective against the risk of cancer in the Chinese and American populations, no relationship was found between this allele and disease susceptibility in the Russian and Caucasian populations, as in our study population (20). The association of rs1052576 with primary brain tumors has been investigated by another study in the Turkish population. Their results showed that the A allele of the Q221R variant decreased the risk for glioma development. In addition, they showed a protective role of *CASP9* SNP rs1052576 GG genotype against gliomas (21). However, Ozdogan et al. did not report the number of glioblastoma cases within the glioma group. In this respect, this study differs from our research. Although the same population and the same disease were studied, the clearest explanation for the different results may be that we examined only glioblastoma patients. Our study reveals more detailed information about whether the SNP is a distinctive genetic factor or not in glioblastoma, a high-grade glioma, in the Turkish population.

Though the link of rs1052576 polymorphism with cancer susceptibility has been documented in these studies, no significant relationship has been found between the *CASP9* SNP rs1052576 and glioblastoma in our study. This controversy with our results might depend on factors like population and ethnic differences.

Consequently, the present study ascertains that *CASP9* SNP rs1052576 is not associated with glioblastoma susceptibility in the Turkish population. However, our study is one of the few studies examining the *CASP9* SNP rs1052576 and glioblastoma susceptibility. The results need to be validated in other independent cohorts, with larger sample sizes.

CONCLUSION

As described earlier, *CASP9* SNP rs1052576 has not been found to be related to glioblastoma. Briefly, these results do not support the role of the *CASP9* rs1052576 variant in susceptibility to glioblastoma disease in the Turkish population.

Ethical Committee Approval: The ethical standards of the 1975 Declaration of Helsinki guidelines and its later amendments were adhered to in all procedures performed in studies involving human participants. The research on humans study protocol was approved by the Yeditepe University Medical Faculty Ethics Committee (file no: 24.10.2018/916).

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