

Role of Caspase-9 Gene Ex5+32 G>A (rs1052576) Variant in Susceptibility to Primary Brain Tumors

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Abstract. *Background/Aim:* This study is the first to evaluate the relationship of caspase-9 (CASP-9) gene polymorphism with the risk for primary brain tumor development. *Materials and Methods:* The study group included 43 glioma and 27 meningioma patients and 76 healthy individuals. CASP-9 gene Ex5+32 G>A (rs1052576) polymorphism was analyzed by real-time polymerase chain reaction (RT-PCR). *Results:* Individuals with the CASP-9 GG genotype had significantly decreased risk of developing a glioma brain tumor ($p=0.024$). Additionally, the GA genotype was significantly lower in patients with glioma than the control group ($p=0.019$). A significantly decreased risk of developing glioma was found in the A allele carrier group ($p=0.024$). However, there was no statistically significant relationship between CASP-9 polymorphism and brain meningioma ($p=0.493$). *Conclusion:* CASP-9 (rs1052576) mutant A allele seems to be a protective factor for glioma brain tumor. Future studies with a larger sample size will clarify the possible roles of CASP-9 gene in the etiology and progression of primary brain tumors.

Primary brain tumors are multifactorial diseases with poor survival (1). The most common types of adult primary brain tumors are gliomas and meningiomas (2). Gliomas account for almost 80% of primary brain tumors and have poor

prognosis despite the use of multimodality treatments, total surgical resections and adjuvant therapies. Gliomas are locally invasive, rarely metastasizing tumors (3, 4). Meningiomas are usually slow-growing benign tumors arising from the meninges (5). Many environmental and lifestyle factors, including smoking, diet, alcohol, exposure to electromagnetic fields, ionizing radiation and several occupations are thought to be associated with increased primary brain tumors risk (6, 7). Researches in molecular medicine have revealed that malignant behavior of primary brain tumors are based on genetic and biochemical abnormalities (8-10). The molecules/pathways identified in these studies could be potential targets for therapeutics.

Abnormal cell growth and proliferation in cancer could be the result of defects in apoptosis (11, 12). Caspases are cysteine proteases which are responsible for diverse cellular functions and apoptosis. There are two major apoptotic pathways known as intrinsic and extrinsic pathways (13). 03-8 and -9 have been proved to be the main caspases in those pathways (14). There are two types of caspases: apoptotic caspases and inflammatory caspases (caspase-1, -4, -5, and -11). Caspase-2, -8, -9, -10, and -12 are known as initiators and caspase-3, -4, -7, and -12 as effectors directly activating the downstream of the initiator caspases, or indirectly activating due to a secondary messenger mechanism, and cleaving certain cellular substrates to cause demolition of the cells (12, 15).

Caspase-9 is a cysteine peptidase encoded by the CASP-9 gene located on chromosome 1p36.1 (16-18). Several candidate and novel polymorphisms in the CASP-9 gene have been recently reported in the databases (19). Nevertheless, the functional effects of those polymorphisms have not been clarified, and it has been assumed that some of the variants can influence CASP-9 expression or activity, thus modulating susceptibility to cancer.

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Therefore, in this study, we conducted a case-control study in a Turkish population, and investigated the association between *CASP-9* Ex5+32 G>A (rs1052576) polymorphism and development of primary brain tumors. To our knowledge, this is the first analysis of the *CASP-9* gene variant in glioma and meningioma patients.

Materials and Methods

Study population. The hospital-based prospective case-control study included 70 primary brain tumors (43 glioma and 27 meningioma). All participants were selected in the Neurosurgery Departments of Kartal Training and Research Hospital and Cerrahpasa University, Istanbul, Turkey. Pathological investigations of brain tumors were determined according to the World Health Organization Classification of Tumors (20). A total of 76 healthy subjects were selected for the control group. The clinical data of the patients were recorded and followed-up prospectively. Demographic characteristics of patients and controls were obtained from medical records of the subjects.

Genetic analysis. After obtaining informed consent from all individuals, peripheral blood samples were collected into EDTA-tubes. DNA extraction was performed by iPrep Purification Instrument (Invitrogen, Life Technologies, Carlsbad, California, USA) by using 350 µl of peripheral blood and Invitrogen iPrep PureLink gDNA blood isolation kit (Invitrogen, Life Technologies, Carlsbad, California, USA). Isolated DNA samples were measured with NanoDrop 2000 (Thermoscientific, Waltham, Massachusetts, USA), 1.7-1.9 optical density range were taken for genotyping and final concentrations of samples diluted to approximately 100 ng/µl. Genotyping for *CASP9* gene rs1052576 polymorphism was performed by Applied Biosystems 7500 Fast Real-Time PCR instrument (Applied Biosystems, Foster City, CA, USA) by using TaqMan Genotyping Assay, TaqMan Genotyping Master Mix (TaqMan Reagents, Applied Biosystems, Foster City, CA, USA) and 100 ng of sample DNA. Reaction mixture and conditions were used as recommended by manufacturer. The reaction conditions were 10 min at 95°C hold stage and 40 cycles of 15 sec at 92°C denaturation and 60 sec at 60°C annealing/extension. Allelic discrimination of samples by collecting and interpreting fluorescent signals of hybridization probes by software of 7500 Fast Real-Time PCR instrument.

Statistical analysis. Statistical analysis were performed using SPSS Ver. 23 software (SPSS Inc, Chicago, IL, USA). The significant differences between groups were examined by Student's *t*-test and demographic informations were compared by Chi square and Fisher's exact tests. $p < 0.05$ was denoted as statistically significant.

Results

The analysis included 43 glioma, 27 meningioma patients and 76 controls. The mean age of the patients with glioma, meningioma and healthy controls were 46.73 ± 10.87 , 52.00 ± 11.61 and 51.44 ± 17.61 years, respectively. No significant differences were found between primary brain tumor and control groups in terms of median age ($p = 0.124$; $p = 0.910$). The frequency of gender was considerably different for the patients and controls (51.4% male, 48.6%

female for patients; 76% male, 24% female for controls). There were significant differences with regards to gender in the study group ($p = 0.002$).

The allele and genotype frequencies for *CASP-9* Ex5+32 G>A (rs1052576) polymorphism in patients with glioma and controls are shown in Table I. *CASP-9* Ex5+32 G>A genotype frequencies between glioma patients and controls were statistically significant ($\chi^2 = 6.305$; $p = 0.043$). As shown in Table I, the frequency of the GG and GA genotype was significantly higher in the control group than the glioma patients ($\chi^2 = 6.305$, $p = 0.024$; OR = 0.363, 95%CI = 0.148-0.890 and $\chi^2 = 5.511$; $p = 0.019$; OR = 0.655, 95%CI = 0.466-0.922). Although there was no significant difference in G allele frequency between the study groups ($\chi^2 = 0.106$; $p = 0.744$), the frequency of A allele was statistically significantly higher in the glioma group ($\chi^2 = 5.511$; $p = 0.024$). Our results indicated that carrying A allele decreased the glioma risk 0.7 fold (OR = 0.754, 95%CI = 0.599-0.948).

The allele and genotype frequencies of *CASP-9* Ex5+32 G>A (rs1052576) in patients with meningioma and controls are given in Table II. GG, GA, and AA genotypes of the patients with meningioma were 25.9%, 51.9% and 22.2%, respectively, and the control subjects were 38.7%, 42.7%, and 18.7%, respectively. The observed genotype frequencies of *CASP-9* Ex5+32 G>A (rs1052576) in patients with meningioma and control groups were in agreement with the Hardy-Weinberg equilibrium ($\chi^2 = 1.412$; $p = 0.493$). In addition, there was no significant difference in *CASP-9* Ex5+32 G>A alleles between the meningioma and control groups ($p > 0.05$).

Discussion

Understanding the molecular mechanisms underlining primary brain tumors assist to cover the gaps in apprehending the pathogenesis of this tumor and potentially provide better prognosis. Several studies have established that some genetic variants affect the expression or the activities of various enzymes and are therefore associated with the cancer risk (21, 22).

Apoptosis is a physiological process regulating programmed cell death (23). Defects in this mechanism can lead to abnormal cell growth and proliferation in cancer development (24). Caspase-9 is a member of the caspases (cysteine-aspartic protease) family involved in the apoptotic process (25). Although some studies have investigated the association of *CASP-9* gene SNPs with cancer risk, results are not clear enough.

The *CASP-9* (Ex5+32 G>A, rs1052576) polymorphism encodes for a glutamine to arginine amino acid change at codon 221 of the protein (26). The Q221R variant might induce conformational changes in the molecule, and because of that may have functional significance (27, 28). However few studies have evaluated the association between this polymorphism and cancer risk. Hosgood *et al.* examined the

Table I. *CASP-9 Ex5+32 G>A (rs1052576) genotype and allele frequencies in patients with glioma and the control group.*

	Glioma n (%)	Control n (%)	p-Value	OR	95%CI
<i>CASP-9</i> (rs1052576) genotype					
GG	8 (18.6%)	29 (38.7%)	0.024*	0.363	0.148-0.890
GA	28 (65.1%)	32 (42.7%)	0.019*	0.655	0.466-0.922
AA	7 (16.3%)	14 (18.7%)	0.744	0.847	0.313-2.295
<i>CASP-9</i> (rs1052576) allele					
G	44 (51.1%)	90 (60.0%)	0.744	0.971	0.819-1.152
A	42 (48.9%)	60 (40.0%)	0.024*	0.754	0.599-0.948

N: Number of individuals; OR: odds ratio; CI: confidence interval. *p-Values less than 0.05 denoted statistical significance.

Table II. *CASP-9 Ex5+32 G>A (rs1052576) genotype and allele frequencies in patients with meningioma and the control group.*

	Meningioma n (%)	Control n (%)	p-Value	OR	95%CI
<i>CASP-9</i> (rs1052576) genotype					
GG	7 (25.9%)	29 (38.7%)	0.235	1.491	0.742-2.999
GA	14 (51.9%)	32 (42.7%)	0.411	0.823	0.526-1.288
AA	6 (22.2%)	14 (18.7%)	0.690	0.840	0.359-1.964
<i>CASP-9</i> (rs1052576) allele					
G	28 (43.7%)	90 (60.0%)	0.690	0.803	0.274-2.359
A	36 (56.3%)	60 (40.0%)	0.235	1.801	0.677-4.791

n: Number of individuals; OR: odds ratio; CI: confidence interval. *p-Values less than 0.05 denoted statistical significance.

association of *CASP-9* Ex5+32 G>A polymorphism with multiple myeloma. They found significantly higher frequencies of AA and AG genotypes in the control group than in patients with multiple myeloma (29). Similarly, Lan *et al.* showed that *CASP-9* rs1052576 polymorphism was significantly associated with decreased risk for non-odgkin lymphoma (30). Zhang *et al.* investigated 2733 neoplastic cases and 3352 healthy controls concerning *CASP-9* Ex5+32 G>A polymorphism. They suggested that the rs1052576 A allele might decrease the risk of cancer (31). Yan *et al.* performed a meta-analysis of 1668 cancer cases and 2294 healthy controls. A allele of Ex5+32 G>A in the *CASP-9* gene was found to have protective factor for cancer risk in Chinese, American and Asian populations, but not in the Caucasian population (32). Conformably, in another meta-analysis, Xu *et al.* showed that the A allele of rs1052576 might be a protective factor for cancer, especially for Asians (33). Previous studies ensured evidence that this SNP may play important roles in the prognosis of cancer in various populations, however is currently unknown for the Turkish population. Additionally, to the best of our knowledge, no published study previously investigated the association between this variant in *CASP-9* gene and the risk of developing primary brain tumors.

Therefore, the present study is the first to evaluate the relevance of *CASP-9* Ex5+32 G>A functional

polymorphism in the risk of glioma and meningioma. Similar to other studies, our results demonstrated that the *CASP-9* rs1052576 A allele was at a decreased risk for glioma development, but no statistical significance for interaction between *CASP-9* gene variant and meningioma was detected. Since genetic polymorphisms often show ethnic differences, further functional studies are needed to evaluate genotype and phenotype correlation in large cohorts of various ethnicities.

Conflicts of Interest

The Authors declare that they have no financial disclosures or conflicts of interest.

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