Abstract. Background: Alteration in cell-cycle control and apoptosis pathways play important roles in tumorigenesis. Caspase-8 (CASP8) is a member of the cysteine protease family, that is implicated in apoptosis regulation. The present study was designed to investigate the possible role of CASP8 D302H gene polymorphism in the tumor development. Materials and Methods: A total of 91 patients with brain tumors (including 39 meningioma and 52 glioma cases) and 114 healthy controls were included in the study. We investigated CASP8 D302H polymorphism by using polymorphism chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. Results: The CASP8 D302H polymorphism genotypic frequencies were not statistically significantly different between meningioma cases and controls, with frequencies of GG, GC and CC genotypes of 71.2%, 19.2% and 9.6%; and 57.9%, 36.8% and 5.3%, respectively. The GG/CC genotypic frequencies were significantly increased in patients with glioma patients compared to controls (p=0.023) (χ²=5.149, odds ratio [OR]=1.27, 95% confidence interval [CI]=1.054-1.551). According to tumor characteristics, there were no statistically significant differences within the groups with astrocytic, oligo-astrocytic tumors and oligodentriogliomas. Conclusion: D302H polymorphism of CASP8 gene may be associated with increased risk of glioma but larger study groups in different ethnic populations are needed to better elucidate the role of CASP8 gene polymorphism in the pathogenesis of primary brain tumors.

Analysis of CASP8 D302H Gene Variants in Patients with Primary Brain Tumors

Correspondence to: Professor Dr. İlhan Yaylım, Istanbul University, Institute for Experimental Medicine Research, Department of Molecular Medicine, Vakif Gureba St., Capa 34390, Istanbul Turkey. Tel: +90 2124142000/33329, e-mail: ilhanyaylim@gmail.com

Key Words: Apoptosis, brain tumors, caspase-8, glioma, gene, genotype, meningioma, polymorphism.

0258-851X/2015 $2.00+.40
radiation therapy treatment had been started. A total of 114 healthy and ethnically matched blood donors served as controls. Informed consent was given from all study participants. Local Ethical Committee approval was obtained for this study from Istanbul Medical Faculty, Project No: 14686.

Isolation of DNA. Blood specimens from all study participants were collected into tubes containing EDTA. Genomic DNA was isolated by sodium dodecyl sulphate lysis, proteinase K digestion, ammonium acetate extraction and ethanol precipitation (13).

CASP8 D302H polymorphism analysis. For genotyping, DNA isolated from the blood of patients and controls was used. CASP8 D302H polymorphism was analyzed using primers 5′-GCTTTGACACGCCTTGAAG-3′ (forward) and 5′-GTTACTGTGGTCCATGAGTTGGTAGAT-3′ (reverse) primer and genotyped by polymorphism chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis, revised according to the method of Engel et al. (7). The PCR reactions were started with an initial denaturation the DNA at 95°C for 15 min, followed by 35 cycles at 94°C for 1 min, 62°C for 1 min, and 72°C for 1 min. The final extension step was at 72°C for 10 min. The PCR products were digested with Hps92II restriction enzyme (MBI Fermentas, Vilnius, Lithuania) at 37°C for >3 h, followed by electrophoresis in 3% agarose gel containing ethidium bromide. The CC genotype site produces bands of 52, 50 and 12 bp; the GG genotype generates two fragments of 102 and 12 bp (7). GC genotype produced four fragments (102, 52, 50 and 12 bp). In the gel, only the 102, 52, and 50 bp fragments were observed.

Statistical analysis. All statistical analyses were carried out using SPSS version 11 for Windows (SPSS Inc, Chicago, IL, USA). Numeric values were analyzed by the Student’s t-test. Differences in characteristics between primary brain tumor cases and controls were assessed with the Chi-square test, as well as disparities in genotypic and allelic frequencies. The Hardy–Weinberg equilibrium was checked with the Chi-square test. Odds ratios (ORs) and 95% confidence intervals (95% CI) were calculated to estimate the risk for brain tumor. p-Values less than 0.05 were considered statistically significant.

Results

This study included 39 meningioma and 52 glioma cases and 114 healthy controls. The clinical characteristics of the study groups are shown in Table I. Genotypic and allelic frequencies of meningioma, glioma cases and controls are listed in Tables II and III.

The genotypic frequencies of CASP8 D302H polymorphism were not statistically significantly different between meningioma cases and controls (p>0.05). The frequencies of GG, GC and CC genotypes in patients with glial tumors and controls were 71.2%, 19.2% and 9.6%; and 57.9%, 36.8% and 5.3%, respectively. Genotypic distributions for CASP8 D302H polymorphism in both groups were in agreement with the Hardy–Weinberg equilibrium (for patients: \( \chi^2=0.064, p=0.79 \); for controls: \( \chi^2=0.036, p=0.848 \)). The homozygous genotypes (GG and CC) were significantly increased in patients with glioma compared to controls (\( \chi^2=5.149, p=0.023 \); OR=1.27, 95% CI=1.054-1.551, \( p<0.05 \)). There were no significant associations between the CASP8 D302H genotypes and clinical parameters such as age, smoking and alcohol consumption, nor pathological parameters such as tumor type, vascular endothelial proliferation or tumor location among patients with brain cancer (data not shown).

In the present study, histological characteristics of glial tumors were heterogeneous. We recorded 29 patients with astrocytoma (63%), while 17 (37%) had glioblastoma multiforme. Among all those with glial tumors, nine (19.6%) had oligodendroglioma, five (10.9%) had oligoastrositoma and three (6.5%) had other types (ependymoma, hemangioblastoma, paranglioma etc.). According to tumor characteristics, there were no statistically significant differences within the groups of patients with astrocytic, oligoastrocytic tumors and oligodentriogliomas (Table IV).

Discussion

Apoptosis, which removes damage cells from organisms, controls cell numbers, tissue size and maintains homeostasis. Caspases have a crucial role as mediators of the apoptotic pathway (14). CASP8 has distinctive features as an initiator death receptor in apoptotic responses (15). According to the literature, the functional effect of D302H polymorphism remains unidentified, but the aspartate residue encoded by CASP8 D302H is conserved in both mouse and Man. The residue has an exposed position on the surface of the protein and for this reason alteration of CASP8 D302H may influence CASP8 interactions with the anti-apoptotic molecule CASP8 FAS-associated protein with death domain-
like apoptosis regulator (CFLAR), or auto-processing of pro-CASP8 molecules (15-20). According to recent studies on independent cohorts, the CASP8 variant D302H may be adversely related with breast cancer risk (9, 19, 20). Yin et al. suggested that CASP8 D302H CC and CG variant genotypes are associated with significantly reduced overall risk of cancer using conservative random genetic models in patients with breast cancer (21). Similarly, MacPherson et al. suggested that CASP8 polymorhism (G>C D302H) was associated with a reduced risk of breast cancer in a dose-dependent manner and individuals with two copies of the H allele had at approximately 40% lower risk of breast cancer compared with those homozygous for the D allele. In the same study, which included 999 patients with breast cancer and 996 controls from Sheffield, UK, they observed that the CASP8 DH and HH genotypic frequencies were statistically significantly lower in cases than in controls (9).

To our knowledge, the present study is the first to evaluate the association between CASP8 D302H polymorphism and brain tumor risk in a Turkish population. We did not observe any significant association for CASP8 D302H genotypic and allelic frequencies between meningioma cases and controls, however, we observed significant differences in the distribution of CASP8 D302H polymorphism in glioma cases. The GG/CC homozygous genotypes were significantly increased patients with glioma compared to controls. Rajaraman et al. reported the Ex14-271A>T (rs13113) and Ex13+51G>C (D285H; rs1045485) polymorphisms in CASP8 were both associated with risk of meningioma but they did not notice any significant association with risk of glioma (22). In another study, Berkhte et al. reported that variation in CASP8 defined by D302H is a determinant of risk and 302H was associated with 1.37-fold increased risk of glioma (6).

Although the present study has some limitations, such as the small size of the study group, our results suggest that the D302H polymorphism in CASP8 gene may be associated with increased risk of glioma. Larger study groups in different ethnic populations are required to better-elucidate the role of CASP8 gene polymorphism in the pathogenesis of primary brain tumors.

References


