RESEARCH ARTICLE

# Investigation of ICAM-1 and β3 Integrin Gene Variations in Patients with Brain Tumors

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## Abstract

**Background: Primary brain tumors constitute a small percent of all malignant cancers, but their etiology remains poorly understood. β3 integrin (ITGB3) has been recognized to play influential roles in angiogenesis, tumor growth and metastasis. Intercellular adhesion molecule-1 (ICAM-1) is a surface glycoprotein important for tumor invasion and angiogenesis. The aim of this study was to investigate whether specific genetic polymorphisms of ICAM-1 and ITGB3 could be associated with brain cancer development and progression in a Turkish population. Our study is the first to our knowledge to investigate the relationship between brain tumor risk and ICAM-1 and β3 integrin gene polymorphisms. Materials and Methods: The study covered 92 patients with primary brain tumors and 92 age-matched healthy control subjects. Evaluation of β3 integrin (Leu33Pro (rs5918)) and ICAM-1 (R241G (rs1799969) and K469E (rs5498)) gene polymorphisms was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Results: According to results of our research, the A allele of the ICAM-1 R241G gene polymorphism appeared to be a risk factor for primary brain tumors (p<0.001). Similarly, the frequency of the A mutant allele of ICAM-1 R241G was statistically significant in patients with brain tumors classified as glioma (p<0.001). When allele and genotype distributions of ICAM-1 K469E, ICAM-1 R241G and β3 integrin Leu33Pro gene polymorphisms were evaluated with age, sex, and smoking, there were no statistically significant differences. Haplotype analysis revealed that the frequencies of GAC (rs1799969-rs5498-rs5918) and GAT (rs1799969-rs5498-rs5918) haplotypes were significantly lower in patients as compared with controls (p=0.001; p=0.036 respectively). Conclusions: This study provides the first evidence that ICAM-1 R241G SNP significantly contributes to the risk of primary brain tumors in a Turkish population. In addition, our results suggest that ICAM-1 R241G in combination ICAM-1 K469E may have protective effects against the development of brain cancer.**

**Keywords:** Cancer - brain tumor - polymorphism - ICAM-1 - β3-integrin

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## Introduction

Brain tumors are composed of a lot of different kinds of cells that constitute the brain tissue and membrane (Stenberg, 1997). Brain tumors are about 2% of all malignant tumors in humans (Fisher et al., 2007). Approximately 75% of all primary brain tumors are classified as glioma or meningioma (Stenberg, 1997). Meningioma generates approximately 25% of all primary intracranial neoplasms and is more commonly seen in middle-aged and elderly patients (Giles et al., 2002). Glioma is one of the most common form of brain tumor that is approximately 12-15% of all intracranial tumors and 50-60% of astrocytic tumors, accounting for creates (Parkin et al., 1998; D'Abaco and Kaye, 2007). Glioma can be seen at any age but is more common, especially in adults (Louis et al., 2000). The incidence of brain tumors shows differences according to gender, age, race, ethnic origin, and geography (Stenberg, 1997). The etiology of brain tumors is still poorly understood. Some studies have reported that environmental exposures and genetic alterations may lead to primary brain tumors development (Wrensch et al., 2005; Fischer et al., 2007; Gu et al., 2009).

Integrins are transmembrane adhesion molecules that are composed of non-covalently linked heterodimeric α and β chains (Sims and Dustin, 2002). For functional activity of the molecule are required both subunits, but the molecule binding specifity is associated with β subunit. By the possession of β subunit structures of β1 integrins, β2 and β3 integrins are called (Sims and Dustin, 2002). β3 integrin gene has 15 exons and covering an area of 60 kb located on chromosome 17q21.3 (Weiss et al., 2006). β3 Integrins are probably required in tumor growth and metastasis (Felding-Habermann et al., 2002; Trikha et al., 2002). The increased expression of cell surface integrins on cancer cells is related to the development of invasive properties, i.e., altered migration on and adhesion to the extracellular matrix (Hood and Cheresh,

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2002). Furthermore, increased integrin expression has also been reported on angiogenic non-neoplastic vascular endothelial cells, assuring the necessary vascular supply for the tumor (Bojesen et al., 2003). Several polymorpisms have been identified in the β3 integrin subunit. A single-nucleotide polymorphism at codon 33 of the β3 integrin gene that exchanges a leucine (Leu) for a proline (Pro) (Leu33Pro/T1565C) seems to modify the function of cells expressing β3 integrins. This polymorphism has been reported to increase activation the mitogen-activated protein kinase (MAPK) (Vijayan et al., 2003) which include another possible functional pathway of increased proliferation of tumor cells.

The ICAM-1 gene, located on chromosome-19q 13.3, consists of seven exons separated by six introns (Dietrich, 2002; Lee, 2004). ICAM-1 is a 76-115 kDa surface glycoprotein which has five extracellular immunoglobulin-like domains (Dietrich, 2002). ICAM-1 is expressed by vascular endothelial cells and leukocytes induced immunoglobulin supergene family of cell adhesion glycoprotein (Dietrich, 2002). Whilst soluble form of ICAM-1 (sICAM-1) is partly detectable in the serum of healthy subjects, its level increases in malignancies. sICAM-1 serum level is associated with tumor size, lymph node and distant metastasis of cancers, including breast cancer, pancreatic cancer, gastric cancer, lung cancer and hepatocellular carcinoma (Nakata et al., 2000; Felding-Habermann et al., 2001; Alexiou et al., 2003; Moriyama et al., 2006; Gogali et al., 2010; Brand et al., 2011; Eggeman et al., 2011). Gliomas call for disruption of endothelial cell-cell attachment and cell-matrix adhesion, cell migration, and formation of new cell–cell interactions. The importance of adhesion molecules in the complex process of tumor development, invasion, metastasis, and interaction with immune cells has been demonstrated in many studies (Demuth and Berens, 2002; Kargiotis et al., 2006). ICAM-1 role on this process and assist the local infiltrative ability of gliomas behavior. Although ICAM-1 expression was shown in high-grade gliomas, its expression was demonstrated weakly expression in low-grade gliomas and no expression in normal brain (Burim et al., 2009). The ICAM-1 gene has at least two biallelic polymorphisms in its coding region. First encoding region Arg/Gly is located at codon 241 (R241G) and the other encoding region Lys/Glu at codon 469 (K469E) (Lee et al., 2004). K469E polymorphism has been found to affect the splicing ICAM-1 mRNA (Iwao et al., 2004; Thanopoulou et al., 2012). This polymorhisms has been studied in various types of malignancies with disparate conclusions (Macchioni et al., 2000; Nakata et al., 2000; Salvarani et al., 2000; Alexiou et al., 2003; Vigano et al., 2003; Lee et al., 2004; Theodoropoulos et al., 2006; Vinceti et al., 2006; Brand et al., 2011; Eggeman et al., 2011). To the best of our knowledge, no published study has investigated the role of polymorphisms of the ICAM-1 and ITGB3 genes on the risk of primary brain tumors.

ICAM-1 K469E, ICAM-1 R241G and β3 integrin Leu33Pro polymorphisms may be changing the function of cells expressing ICAM-1 and β3 integrins. Also these polymorphisms may be affecting cancer progression, thus we hypothesized that the ICAM-1 (K469E), (R241G) and β3 integrin subunit Leu33Pro polymorphism could influence cancer risk.

## Materials and Methods

*Subjects*

We investigated the β3 integrin Leu33Pro, ICAM-1 K469E and ICAM-1 R242G gene polymorphisms in 92 brain cancer patients (including 54 cases with glioma and 38 cases with non-glioma brain tumors) and 92 healthy control matched subjects who were in the follow-up Cerrahpasa Faculty of Medicine, Department of Neurosurgery in Istanbul University. The mean ages of brain cancer patients and control group were 47.37±14.67 and 48.12±14.79 years, respectively.

The specimens were taken after obtaining informed consent and the study was conducted prospectively. Medical Ethics Committee of Istanbul Medical Faculty approval was obtained for the study. The protocol followed was consistent with the World Medical Association Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects).

*Genotyping*

DNA was extracted from whole blood using Pure Link DNA extraction kit (Invitrogen, USA). The DNA concentration was determined by measuring the OD at 260 nm. Genotyping was performed by the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). For β3 Integrin Leu33Pro polymorphism, the following primers were used to amplify the β3 Integrin gene; sense primer 5'-GCT CCA ATG TAC GGG GTA AAC-3' ; antisense primer 5'-GGG GAC TGA CTT GAG TGA CCT-3'. For detection of the β3 Integrin Leu33Pro RFLP, 20 ng genomic DNA was amplified with 1 X PCR buffer, 3 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 mM of each primer and Taq polymerase (Fermantas, Lithuania) in a 25 $\mu$l reaction volume. The PCR conditions involved an initial denaturation of 5 min at 95ºC, followed by 35 cycle of 94ºC 45 sec, annealing at 59ºC for 45 sec and extension 72ºC for 45 sec. A final extension step at 72ºC for 5 min was also studied. PCR products were digested with MspI (Fermentas, Lithuania) restiriction enzyme at 37ºC for 16 h and electrophoresised on 3% agarose gels and stained with ethidium bromide. Genotypes were determined as TT (282 bp), TC (282,157,125 bp) or CC (157,125 bp) for β3 Integrin Leu33Pro polymorphism. The primers for ICAM-1 K469E polymorphism were sense 5'-GGA ACC CAT TGC CCG AGC-3'; antisense 5'-GGT GAG GAT TGC ATT AGG TC-3'. The DNA template was amplified by PCR using 3 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 mM of each primer and Taq polymerase (Fermentas, Lithuania) in a 25 $\mu$l final volume. The PCR conditions involved an initial denaturation of 5 min at 95ºC, followed by 35 cycles of 94ºC for 45 sec, annealing at 57ºC for 45 sec and extension at 72ºC for 45 sec. A final extension step at 72ºC for 5 min was also studied. PCR products were digested with BstUI (Fermantas, Lithuania) restiriction enzyme at 37ºC for 2 h followed by electrophoresis in a

3 % agarose gel. Genotypes were determined as AA (223 bp), AG (223,137,87 bp) or GG (136,87 bp) for ICAM-1 K469E polymorphism. The oligonucleotide primers to amplify region of ICAM-1 R241G were: 5'-CCG TGG TCT GTT CCC TGT AC-3' (sense), 5'-GAA GGA GTC GTT GCC ATA GG-3' (antisense) respectively. PCR was performed in a total volume of 25 μl with 0.3 μl Taq DNA polymerase (Fermantas, Lithuania). The PCR mix was incubated for 5 min at 95ºC followed by 35 cycles of 45 sec at 94ºC, 45 sec at 57ºC for ICAM-1 R241G polymorphism. PCR products were digested with BsrGI (Fermantas, Lithuania) restiriction enzyme at 37ºC for 16 h followed by electrophoresis in a 3% agarose gel. Genotypes were determined as GG (110 bp), AA (90, 20 bp) or GA (110, 90, 20 bp) for ICAM-1 R241G polymorphism.

*Statistical Analysis*

Statistical analysis were performed using SPSS version 11.5 (SPSS Inc, Chicago, USA) including the Chi-square ($\chi^2$) test, Fischer's exact test and the Pearson correlation test, odds ratio (OR) and 95% confidence intervals (Cl) were calculated. Mean values were compared among subjects by the unpaired Student's t-test. Haplotype analysis was undertaken using Haploview 4.2. software program. Values of $p<0.05$ were considered as statistically significant.

## Results

*Subject characteristics*

The analysis included 92 brain cancer (including 54 cases with glioma and 38 cases with non-glioma brain tumors) and 92 healthy controls. Informations on study groups are shown in Table 1. There was no significant difference statistically between brain tumor cases and controls in terms of age and gender parameters (p=0.848; p=0.184, respectively).

*Association between ICAM-1 and β3 integrin polymorphisms and risk of brain tumors*

Genotype and allele frequencies of brain tumor cases and controls are shown in Table 2. Firstly, we evaluated association between ICAM-1 R241G polymorphism and study groups. The frequency of the A mutant allele was significantly higher patients compared to controls ($\chi^2$=15.311, p<0.001, respectively). When ICAM-1 K469E and β3 Integrin Leu33Pro polymorphisms genotype and allele frequencies compared in study groups, no statistically significant difference was detected (p>0.05).

In the present prospective case-control study, the primary brain tumors were divided into two groups, namely glioma with a malignant tumors and namely meningioma with benign tumors. Genotype and allele distributions of ICAM-1 K469E and R241G SNPs were statistically significant in patients with brain tumors classified as glioma. Frequency of A wild type allele for ICAM-1 K469E was significantly lower in glioma patients compared to the control group ($\chi^2$=4.089, p=0.043), (Table 3). However, frequency of A mutant allele for ICAM-1 R241G was significantly higher in glioma patients compared to the control group ($\chi^2$=16.341, p<0.001), (Table 3). The observed genotype frequencies of ICAM-1 and β3 Integrin polymorphisms between meningioma and control group were in agrement with Hardy-Weinberg equilibrium (p>0.05). When allele frequencies of these polymorphisms compared between meningioma and control groups, no statistically significant difference was detected (p>0.05).

According to the grade of tumor patients with brain tumors are grouped as grade I-II and grade III-IV, genotype GG for ICAM K469E were significantly higher than AA+AG genotype in patients with high grade (III+IV)

**Table 1. Characteristics of the Study Groups**

| | | Control (n=92) | Patient (n=92) | p value |
|---|---|---|---|---|
| Gender | Female | 56.5% | 46.7% | 0.184 |
| | Male | 43.5% | 53.3% | |
| Average of age (year) | | 48.12±14.79 | 47.37±14.67 | 0.848 |

*p values less than 0.05 denoted statistical significance; **n=number of individuals, Chi-square test was used to compare gender and average of age in the study group

**Table 2. Genotype and Allele Frequencies of Patients with Brain Tumors and Controls**

| Genotypes and Alleles | | Patient n (%) | Control n (%) | p value |
|---|---|---|---|---|
| ICAM-1 K469E | AA | 25 (27.2) | 29 (31.5) | |
| | AG | 49 (53.3) | 50 (54.3) | 0.479 |
| | GG | 18 (19.5) | 13 (14.2) | |
| | A allele | 99 (53.8) | 108 (58.70) | |
| | G allele | 85 (46.19) | 76 (41.30) | 0.344 |
| ICAM-1 R241G | GG | 78 (84.8) | 92 (100) | |
| | GA | 7 (7.6) | 0 (0) | 0.004* |
| | AA | 7 (7.6) | 0 (0) | |
| | G allele | 163 (88.59) | 184 (100) | |
| | A allele | 21 (11.41) | 0 (0) | 0.001* |
| β3 Integrin Leu33Pro | CC | 5 (5.4) | 1 (1.1) | |
| | TC | 12 (13.0) | 12 (13.0) | 0.561 |
| | TT | 75 (81.5) | 79 (85.9) | |
| | C allele | 22 (11.96) | 14 (7.61) | |
| | T allele | 162 (88.04) | 170 (92.39) | 0.160 |

*p values less than 0.05 denoted statistical significance; **n=number of individuals, Chi-square test was used to compare genotypes and alleles characteristics in the study group

**Table 3. Genotype and Allele Frequencies of Meningioma and Glioma Patients and Controls**

| | Genotypes and Allele | Meningioma n (%) | Glioma Controls n (%) | |
|---|---|---|---|---|
| ICAM-1 K469E | AA | 15 (39.5) | 10 (18.5) | 29 (31.5) |
| | AG | 20 (52.6) | 29 (53.7) | 50 (54.3) |
| | GG | 3 (7.9) | 15 (27.8) | 13 (14.1) |
| | A allele | 50 (65.29) | 49 (45.37)* | 108 (58.70) |
| | G allele | 26 (34.21) | 59 (54.63) | 76 (41.30) |
| ICAM-1 R241G | GG | 33 (86.8) | 45 (83.3) | 92 (100) |
| | GA | 3 (7.9) | 4 (7.4) | 0 (0) |
| | AA | 2 (5.3) | 5 (9.3) | 0 (0) |
| | G allele | 69 (90.79) | 94 (87.03) | 184 (100) |
| | A allele | 7 (9.21) | 14 (12.97)** | 0 (0) |
| β3Integrin Leu33Pro | TT | 34 (89.5) | 41 (75.9) | 79 (85.9) |
| | TC | 3 (7.9) | 9 (16.7) | 12 (13.0) |
| | CC | 1 (2.6) | 4 (7.4) | 1 (1.1) |
| | T allele | 71 (93.42) | 91 (84.26) | 170 (92.39) |
| | C allele | 5 (6.58) | 17 (15.74) | 14 (7.61) |

*p=0.043; glioma patients compared to controls; **p<0.001; glioma patients compared to controls; #n=number of individuals, Chi-square test was used to compare genotypes and alleles characteristics in the study group. p values less than 0.05 denoted statistical significance

**Tablo 4. According to the Grade of Tumor Patients with Brain Tumors are Grouped as Grade I-II and Grade III-IV**

| Genotypes | | Patients with grade I-II tumors n (%) | Patients with grade III-IV tumors n (%) | OR 95% (CI) | p value |
|---|---|---|---|---|---|
| ICAM-1 K469E | AA+AG | 15 (93.8) | 24 (63.2) | 0.674 (0.512-0.886) | *0.022 |
| | GG | 1 (6.3) | 14 (36.8) | 5.895 (0.845-41.137) | |
| | GG+AG | 13 (81.3) | 31 (81.6) | 1.004 (0.759-1.328) | 0.977 |
| | AA | 3 (18.8) | 7 (18.4) | 0.982 (0.290-3.327) | |
| | AA+GG | 4 (15) | 21 (51.3) | 2.211 (0.903-5.413) | **0.042 |
| | AG | 12 (75) | 17 (44.7) | 0.596 (0.379-0.938) | |
| ICAM-1 R241G | GG+GA | 16 (100) | 33 (86.8) | 0.868 (0.767-0.983) | 0.128 |
| | AA | 0 (0) | 5 (13.2) | | |
| | GA+AA | 2 (12.5) | 7 (18.4) | 1.474 (0.343-6.339) | 0.584 |
| | GG | 14 (87.5) | 31 (81.6) | 0.932 (0.734-1.184) | |
| | GA | 2 (12.5) | 2 (5.3) | 0.421 (0.065-2.734) | 0.354 |
| | GG+AA | 14 (87.5) | 36 (94.7) | 1.083 (0.887-1.322) | |
| β3Integrin Leu33Pro | TT+TC | 16 (100) | 34 (89.5) | 0.895 (0.802-0.998) | 0.177 |
| | CC | 0 (0) | 4 (10.5) | | |
| | TC+CC | 3 (18.8) | 10 (26.3) | 1.404 (0.444-4.434) | 0.533 |
| | TT | 13 (81.3) | 28 (73.7) | 0.907 (0.670-1.227) | |
| | TT+CC | 13 (81.3) | 32 (84.2) | 1.036 (0.789-1.361) | 0.790 |
| | TC | 3 (18.8) | 6 (15.8) | 0.842 (0.240-2.959) | |

*GG genotype in patients with III. ve IV. grade tumors; **AA+GG genotype in patients with III. ve IV. grade tumors; #Chi-square test was used to compare genotypes and alleles characteristics in the study group.n, number of individuals. p values less than 0.05 denoted statistical significance

**Table 5. Haplotype Frequencies of the ICAM-1 and β3 Integrin Polymorphisms Among the Patients and Controls**

| Haplotype association | Frequency | Case; Control Ratio Counts | Chi square | p value |
|---|---|---|---|---|
| AGT | 0.527 | 100.4: 93.6, 94.5: 81.5 | 0.14 | 0.707 |
| GGT | 0.356 | 63.4: 130.6, 68.5: 107.5 | 1.558 | 0.212 |
| GGC | 0.037 | 6.2: 187.8, 7.5: 168.5 | 0.302 | 0.582 |
| GAC | 0.029 | 10.7: 183.3, 0.0: 176 | 9.949 | 0.001* |
| AGC | 0.023 | 3: 191, 5.5: 170.5 | 1.022 | 0.311 |
| GAT | 0.013 | 4.7: 189.3, 0.0: 176 | 4.364 | 0.036* |

*p values less than 0.05 denoted statistical significance; **Haplotype consist of rs1799969 (ICAM-1 R241G), rs5498 (ICAM-1 K469E) and rs5918 (β3Integrin Leu33Pro). p values of haplotype association were calculated using Haploview 4.2

compared patients with lower grade (I+II) (p=0.022), (Table 4). However, genotype AA+GG for ICAM K469E was significantly higher than AG genotype in patients with high grade (III+IV) compared patients with lower grade (I+II) (p=0.042), (Table 4).

Allele and genotype distributions of ICAM-1 K469E, ICAM-1 R241G and β3 Integrin Leu33Pro polymorphisms were evaluated with age, sex, and smoking, there was no statistically significant difference (p>0.05). Genotype combinations of ICAM-1 K469E, ICAM-1 R241G and β3 Integrin Leu33Pro polymorphisms were examined, a statistically significant difference was not found between the carrying combined genotype in patients with brain tumors and the control group (p>0.05).

*Haplotype analysis between ICAM-1 and β3 integrin polymorphisms in each groups*

In addition to SNP analyses, haplotypes were evaluated for association with brain cancers. Haplotype analysis revealed that the frequencies of K469E G: R241 A: Leu33Pro C haplotype was significantly lower in patients as compared with those of controls ($\chi^2$=9.949, p=0.001). However, haplotype analysis showed that the frequencies of K469E G: R241 A: Leu33Pro T haplotype was significantly lower in patients than in the controls ($\chi^2$=4.364, p=0.036). There was no relationship between other haplotypes and brain cancers (D' 0.526, LOD:1.51, r-squared:0.022), (Table 5).

## Discussion

Cancer is the abnormal cell growth that causes cell proliferation and morbidity due to multiple gene expressions, and if not treated, it may result in tissue invasion and metastasis leading to dead (Ruddon, 2007). Despite primary brain tumors are less than 2% of all malignancies, primary brain tumors have been reported which are second only to stroke as a cause of death from neurologic disorders (Burgess, 2010). Brain tumors are graded on the basis of histopathology into the following main histologic groupings: neuroepithelial tissue tumors such as glioma, including astrocytoma (grade II), anaplastic astrocytoma (grade III), glioblastoma (grade IV), oligodendroglioma, and ependymoma, meninges tumors, germ cell tumors, and sellar region tumors including pituitary tumors and craniopharyngioma (Brooks et al., 1994).

β3 Integrin play a role on the pathophysiology of malignant tumors. It is implicated on endothelial cells, where β3 Integrin is involved in tumor angiogenesis (Tian et al., 2012). The tumor cells express β3 Integrin and this expression has been shown in several malignancies. β3 Integrin expression is associated with tumor progression in melanoma, glioma, and ovarian and breast cancer (Kammerer et al., 2004). ICAM-1 has a key role in cell-to-cell, cell-to-extracellular matrix interaction and cell signaling. Some studies indicate which ICAM-1 has an important role in the development and progression of human cancers. Overexpression of ICAM-1 in melanoma, gastric, breast, renal cell carcinoma and colorectal cancer

has been shown (Arandi et al., 2008). The SNP in ICAM1 gene has been also reported which is correlated with the risk of cancers, such as breast cancer, colorectal cancer and melanoma (Howell et al., 2005; Cox et al., 2006; Vinceti et al., 2006; Wang et al., 2009).

In our study, genotype and allele distribution of ICAM K469E were not observed statistically significant difference between patients with brain tumors and control group. Similarly, Vigano et al. (2003) have not found a significant relationship for ICAM K469E polymorphism allele and genotype frequencies between endometrium patients and the control group. At the same time, Arandi et al. (2008) have reported that distribution of ICAM K469E polymorphism allele and genotype was not statistically significant difference between breast cancer and control. However, AA genotype for ICAM-1 K469E has been shown that increased cancer risk in colorectal cancer (Wang et al., 2009), stomach cancer (Tian et al., 2012) and the colon (Theodoropoulos et al., 2006) cancer studies. Our results also demonstrated that genotype and allele distributions of ICAM R241G were found statistically significant differences between the control and patient groups. Arandi et al. (2008) have determined statistically significant of GA genotype frequency compared with GG and AA genotypes for ICAM R241G in breast cancer patients. Also, same results were obtained in endometrium (Vigano et al., 2003) and colon cancer (Theodoropoulos et al., 2006) studies. Genotype distribution of β3 Integrin Leu33Pro polymorphism was not statistically significant between patients and controls in breast cancer studies (Wang-Gohrke et al., 2005; Langsenlehner et al., 2006). Bojesen et al investigated relationship between β3 Integrin Leu33Pro gene polymorphism and 27 cancer types in 9242 cancer patients and they have reported that β3 Integrin Leu33Pro gene polymorphism is not a risk factor for cancer (Bojesen et al., 2003). Analogously, β3 Integrin Leu33Pro SNP was not associated with primary brain tumors development in our case-control study.

In the present prospective case-control study, the primary brain tumors were divided into two groups, namely gliomas with a malignant tumors and namely meningiomas with benign tumors. Frequency of A mutant allele of ICAM-1 R241G were statistically significant in patients with brain tumors classified as glioma. Gliomas are common tumors and account for almost 80% of primary malignant brain tumors, usually resulting in poor survival compared to other types of brain tumors.

In addition to SNP analyses, haplotypes were evaluated for association with primary brain tumors. Haplotype analysis suggested that primary brain tumor risk was highly reduced among individuals with spesific haplotypes. Haplotype association analysis showed that GAC (ICAM-1 K469E G allele: ICAM-1 R241 A allele: β3 Integrin Leu33Pro C allele) and GAT (ICAM-1 K469E G allele: ICAM-1 R241 A allele: β3 Integrin Leu33Pro T allele) haplotype was associated with the risk of primary brain tumors in our study. The combination of ICAM-1's SNPs may have protective effect on the risk of brain tumors.

In conclusion, our findings provides new evidence for the association between ICAM-1 R241G SNP and the risk of primary brain tumors in a Turkish population. These findings also suggest that ICAM-1 R241G gene polymorphism might affect the development of glioma tumors. At the same time, we identified a previously undescribed ICAM-1 R241G and ICAM-1 K469E haplotype in association with the primary brain tumor in our population. The haplotype of ICAM-1 K469E mutant G: ICAM-1 R241 mutant A may be a protective marker for genetic susceptibility to primary brain tumors. Our results needs to be confirmed in larger cohorts in order to better understand their role in development of brain cancer

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