

Role of *SOD2* Ala16Val polymorphism in primary brain tumors

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Abstract. The present study aimed to investigate the possible association between the genetic polymorphism of the enzyme superoxide dismutase 2 (SOD2, also known as manganese-dependent SOD), Ala16Val (rs4880), and primary brain tumor risk in the Turkish population. Frequency of the *SOD2* gene rs4880 polymorphism was identified in 225 Turkish individuals (120 controls and 105 patients with primary brain tumor) by polymerase chain reaction-restriction fragment length polymorphism. Subject demographics and clinical characteristics were also recorded. The findings were evaluated using logistic regression and χ^2 tests. Logistic regression analysis indicated that smoking did not increase the risk for primary brain tumor [odds ratio (OR)=0.77, 95% confidence interval (CI)= 0.44-1.33, $\chi^2=0.352$, $P=0.860$]. Similarly, there was no statistically significant difference in the family history of cancer incidence between the control subjects and the primary brain tumor patients (OR=0.81, 95% CI=0.39-1.71, $\chi^2=0.340$, $P=0.560$). There was no significant association of the histopathological type, genotype/allele frequencies and inheritance models of tumor with the gene variants among the patients with primary brain tumor. In summary, the results of the present study indicated that the Ala16Val polymorphism of the *SOD2* gene was not associated with primary brain tumor risk in the Turkish population studied.

Introduction

Cancer is a major public health problem worldwide. Brain and central nervous system tumors refer to a series of rarely-occurring tumors (1). In 2018, it is expected that ~23,880 new cases (13,720 males and 10,160 females) of brain and central nervous system tumors were diagnosed and 16,830 (9,490 males and 7,350 females) are expected to have died due to these types of

tumor in the United States, accounting for approximately 2% of all cancers diagnosed nationally (1,2). Data published by the Turkish Health Ministry in 2018 (Turkey United Database, 2011-2015) indicated that approximately 163,500 new cases of cancer are diagnosed annually, and approximately 450 individuals are diagnosed with cancer daily; furthermore, brain, meningeal and central nervous system tumors in Turkey had a reported prevalence of 5.3/100,000 among males and 4.1/100,000 among females (3,4). Primary brain tumors are a highly heterogeneous group of tumors that are still not fully understood in terms of underlying pathological mechanism. The effect of genes on the development of brain tumors is still poorly understood. However, understanding the genetic risk factors associated with a given cancer is important for selecting treatment (5,6). It has been previously reported that reactive oxygen species (ROS) serve an important role in tumor development (7,8). ROS may arise from intrinsic factors including mitochondria, peroxisomes and inflammatory cell activation, and contact with extrinsic factors including environmental agents, pharmaceuticals and industrial chemicals (9,10). ROS cause oxidative stress, and are typically counterbalanced by antioxidant defenses of the cell (9). Oxidative stress may cause DNA, protein and/or lipid damage, leading to an increased chance of chromosome instability, genetic mutation and/or modulation of cell growth that may result in cancer (11). The association between brain tumors and antioxidant enzyme polymorphisms has been investigated by several studies in the literature (12,13).

Superoxide dismutase 2 [SOD2, also known as manganese (Mn)SOD], as one of the major antioxidant enzymes, catalyzes the dismutation of superoxide radicals to H_2O_2 and oxygen in mitochondria, and thereby constitutes a first-line defense against ROS in mitochondria (13). Thus, it is conceivable that structural and/or functional polymorphisms of the *SOD2* gene have critical impact on the maintenance of ROS levels in cells (13,14). The *SOD2* gene structure consists of five exons interrupted by four introns and the promoter, which control *SOD2* expression (15). Several polymorphisms have been identified in distinct regions of the *SOD2* gene (15). These polymorphisms have been associated with different diseases. *SOD2* is encoded by the nuclear *SOD2* gene located on the human chromosome 6q25 and the Ala16Val polymorphism has been identified in exon 2 of the human *SOD2* gene. Ala16Val (47C>T or rs4880) is a frequently studied *SOD2* single nucleotide polymorphism (SNP) on *SOD2* gene and is known to cause a conformational change (15,16). As

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mentioned, the *SOD2* Ala16Val polymorphism is located in the mitochondrial targeting sequence, and has been suggested to modify the peptide structure, affecting protein translocation and maturation into the mitochondrial matrix (17). This polymorphism has been associated with different pathologies including asthma (18), diabetes (19,20), the aging process (21), cardiomyopathy (22) and cancer (23).

To our knowledge, there has been no study of the association of *SOD2* Ala16Val polymorphism with primary brain tumor susceptibility in a Turkish population. Therefore, in the present study, the aim was to investigate the association between the genetic polymorphism *SOD2* Ala16Val and primary brain tumor risk in a Turkish population.

Materials and methods

Study population. In the present study, a total of 225 Turkish individuals were studied. Cases were brain tumor patients (n=105) who attended the Neurosurgery Department at Cumhuriyet University Hospital in Sivas (Central Anatolia, Turkey). Only the patients with newly diagnosed primary brain tumor and no previous radiotherapy or chemotherapy were included in the study. There was no sex, age or tumor type restriction. The diagnosis of brain tumor tissue removed by surgery was histologically confirmed and the tumor types were classified according to World Health Organization guidelines 2007 (24). Age and sex-matched controls were obtained from patients with no previous cancer diagnosis or radio- or chemotherapy recruited at the same hospital (n=120). All study subjects agreed to participate and completed a short questionnaire including questions on occupation, tobacco use and family history of cancer (Table I). The local Sivas Cumhuriyet University Ethics Committee (Sivas, Turkey) on human research approved the study (approval no. 2003/304). Patients and controls were informed on the study and provided written informed consent.

DNA isolation. Peripheral blood samples (2 ml) were collected into citrate (0.109 M)-containing (BD Vacutainer; BD Biosciences, San Jose, CA, USA) tubes from all subjects. DNA was immediately extracted from whole blood by a salting out procedure as described previously (25).

Genotyping. The distribution of the *SOD2* rs4880 polymorphism was examined using a polymerase chain reaction (PCR)-restriction fragment length polymorphism method. For amplification of the rs4880 polymorphism, the forward primer 5'-ACCAGCAGGCAGCTGGCGCCGG-3' and reverse primer 5'-GCGTTGATGTGAGGTTCCAC-3' were used. The PCR reactions contained 0.75 µl (25 pmol/µl) of each primer, 2 µl dNTPs (1 mmol/l), 1.5 µl of 25 mmol/l MgCl₂, 1.5 units of Taq DNA polymerase (5 U/µl Fermentas; Thermo Scientific, Inc., Pittsburgh, PA, USA) and 16.2 µl sterile deionized water. DNA (50-100 ng) was added to a total volume of 50 µl. The PCR program was initial denaturation at 95°C for 5 min followed by 35 cycles of 95°C for 1 min, 61°C for 1 min (annealing) and 72°C for 2 min (extension). The reaction was completed by a final extension cycle at 72°C for 7 min. Amplified product was digested overnight with *PdiI* restriction enzyme (ER1521, 200 U; Fermentas; Thermo Scientific, Inc.)

Table I. Demographics and medical history of cancer patients and controls.

Controls	Cancer	patients
Sample size	120	105
Sex		
Male	70 (58.3)	61 (58.1)
Female	50 (41.7)	44 (41.9)
Age, years		
Range	14-80	10-82
Mean ± SD	54.41±5.76	51.41±16.20
Males	55.34±5.76	52.20±15.85
Females	53.12±7.16	50.32±16.79
Smoking history		
Smoker	39 (32.5)	40 (38.1)
Non-smoker	81 (67.5)	65 (61.9)
P-value	0.860	
χ ²	0.352	
OR (95% CI) ^a	0.77 (0.44-1.33)	
Family history of cancer		
Yes	23 (19.2)	17 (16.2)
No	97 (80.8)	88 (83.8)
P-value	0.560	
χ ²	0.340	
OR (95% CI) ^b	0.81 (0.39-1.71)	

Data are presented as frequency (%) or as indicated. ^aOR adjusted for age and family history of cancer; ^bOR adjusted for age and smoking. OR, odds ratio; CI, confidence interval; SD, standard deviation.

at 37°C and electrophoresed on 3% agarose gel and stained with ethidium bromide. Genotypes were determined for the polymorphism as TT (107 bp), CT (107, 89 and 18 bp) and CC (89 and 18 bp) (26).

Statistical analysis. All statistical analyses were performed using SPSS version 13.0.1 (SPSS, Inc., Chicago, IL, USA). Genotype-associated odds ratios, their corresponding 95% confidence intervals and associated P values were estimated via unconditional logistic regression. Differences in the distributions of demographic characteristics between the cases and control subjects were evaluated using a Student's t-test, χ² or Fisher's exact test (two-sided) to compare the sex distribution. To determine the association between the genotypes and alleles in relation to the controls and the deviation of the genotype distribution, Hardy-Weinberg equilibrium was employed. Pearson's χ² test was used to determine whether there were any significant differences in allele and genotype frequencies between patients and control subjects. Logistic regression procedures were performed to assess the interaction between age, sex and all genotypes. The association between the *SOD2* Ala16Val polymorphism and primary brain tumors in five inheritance genetic models (codominant, dominant, recessive, overdominant and additive) was assessed. In addition, the recessive or dominant effect of the *SOD2* genotype on risk

Table II. Genotype and allele frequencies in the primary brain tumor and the healthy control groups and association of *SOD2* gene rs4880 polymorphism with the risk of primary brain tumor susceptibility according to different models of inheritance.

	Group, n (%)		OR (95% CI)	χ^2	P-value
	Controls (n=120)	Cancer patients (n=105)			
SOD2 rs4880					
C allele	113 (47.1)	98 (46.7)	1.00 (reference)		
T allele	127 (52.9)	112 (53.3)	1.02 (0.69-1.50)	1.01	0.929
Codominant					
CC	29 (24.2)	25 (23.8)	1.00 (reference)		
CT	55 (45.8)	48 (45.7)	1.01 (0.50-2.07)	0.01	0.970
TT	36 (30.0)	32 (30.5)	1.03 (0.47-2.25)	0.01	0.933
Dominant					
CC	29 (24.2)	25 (23.8)	1.00 (reference)		
CT+TT	91 (75.8)	80 (76.2)	1.02 (0.53-1.97)	0.01	0.978
Recessive					
CC+CT	84 (70.0)	73 (69.5)	1.00 (reference)		
TT	36 (30.0)	32 (30.5)	0.96 (0.52-1.75)	0.02	0.880
Overdominant					
CC+TT	65 (54.2)	57 (54.3)	1.00 (reference)		
CT	55 (45.8)	48 (45.7)	1.00 (0.57-1.74)	0.01	0.986

SOD2, superoxide dismutase 2; OR, odds ratio; CI, confidence interval.

was estimated, and statistical analysis was performed on the relative risk of the CC genotype against the CT + TT genotype (Dominant), the CC + CT genotype against the TT genotype (recessive) or the CC+TT genotype against the CT genotype (overdominant).

Results

Demographics and medical history. The demographic characteristics of the study population are listed in Table I. The mean ages of the patients and controls were 51.41±16.20 and 54.41±5.76 years, respectively. The percentages of males and females in the case group were 58% (61/105) and 42% (44/105), respectively. The mean ages of the men and women patients were similar (52.20±15.85 for men and 50.32±16.79 for women). Although family history of cancer was less prevalent in the cases (16%) than controls (19%), no statistically significant association was determined in the cases compared with the controls (OR=0.81, 95% CI=0.39-1.71, $\chi^2=0.340$, P=0.560). No statistically significant difference was observed between controls (32.5%) and cases (38.1%) regarding smoking status (OR=0.77, 95% CI=0.44-1.33, $\chi^2=0.352$, P=0.860; Table I).

Genotype and allele frequencies. All genotypes and alleles in the controls and cases were in Hardy-Weinberg equilibrium (P>0.05). The frequencies of the TT, CT and CC genotypes were determined to be 30.0 vs. 30.5%, 45.8 vs. 45.7% and 24.2 vs. 23.8% in the controls vs. cases, respectively ($\chi^2=0.01$, P=0.933; $\chi^2=0.01$, P=0.970). The frequencies of C and T alleles were determined to be 47.1 vs. 46.7% and 52.9

Table III. ORs for the association of *SOD2* gene rs4880 polymorphism with primary brain tumor incidence.

Genotype	Group, n (%)	
	Cancer patients (n=105)	Controls (n=120)
CC	25 (23.8)	29 (24.2)
CT + TT	80 (76.2)	91 (75.8)
χ^2	0.01	
P-value	0.978	
Unadjusted OR (95% CI)	1.02 (0.53-1.97)	
Adjusted OR (95% CI) ^a	1.04 (0.54-2.02)	

^aOR adjusted for sex, age, smoking and family history of cancer. SOD2, superoxide dismutase 2; OR, odds ratio; CI, confidence interval.

vs. 53.3%, respectively, in the controls vs. cases (Table II). The analysis of the polymorphism located at *SOD2* chromosome 6 (6q25) (16) in the controls demonstrated TT (107 bp), CT (107, 89 and 18 bp), CC (89 and 18 bp) genotypes.

For the *SOD2* Ala16Val polymorphism, statistically significant associations were not identified in allele frequency (C vs. T: OR=1.02, 95% CI=0.69-1.50, P=0.929), codominant (CC vs. CT: OR=1.01, 95% CI=0.50-2.07, P=0.970; CC vs. TT: OR=1.03, 95% CI=0.47-2.25, P=0.933), dominant (CC vs. CT+TT: OR=1.02, 95% CI=0.53-1.97, P=0.978), recessive

Table IV. Association between *SOD2* gene rs4880 variants and histological type of brain tumor.

Genotype	Histological type of brain tumor (n=105)				
	Controls	Meningioma	Astrocytoma	Hypophysis adenoma	Others
n (%)	120 (100.0)	27 (25.7)	43 (41.0)	14 (13.3)	21 (20.0)
CC	29 (24.2)	5 (18.5)	10 (23.3)	5 (35.7)	6 (28.6)
CT+TT	91 (75.8)	22 (81.5)	33 (76.7)	9 (64.3)	15 (71.4)
χ^2	-	0.40	0.01	0.88	0.19
P-value	-	0.529 ^a	0.941	0.261 ^a	0.666
OR (95% CI)	-	1.40 (0.45-4.66)	1.05 (0.43-2.60)	0.57 (0.16-2.16)	0.80 (0.26-2.55)

^aFisher exact test. SOD2, superoxide dismutase 2; OR, odds ratio; CI, confidence interval.

(CC+CT vs. TT: OR=0.96, 95% CI=0.52-1.75, P=0.880) or overdominant (CC+TT vs. CT: OR=1.00, 95% CI=0.57-1.74, P=0.986) inheritance models (Table II). The *SOD2* polymorphism genotype distributions in controls and cases are given in Table II.

Furthermore, there was no significant association between *SOD2* rs4880 genotype and brain tumor incidence. Distribution of the *SOD2* CC genotype was 23.8% in cases and 24.2% in controls (adjusted OR=1.04, 95% CI=0.54-2.02, P=0.978; Table III).

Histological type of brain tumor. To evaluate the association between the polymorphism of *SOD2* and the specific type of brain tumor, the patients were subdivided according to the histopathological type of the brain tumor. The most prevalent tumor types were meningioma (25.7%), astrocytoma (41.0%) and hypophysis adenoma (13.3%). Others (20.0%) included pituitary adenoma, medulloblastoma, colloid cyst, craniopharyngioma and acoustic neuroma. There was no statistically significant association of any of the tumor types with the polymorphism of the *SOD2* (Table IV).

Discussion

Brain and other central nervous system tumors are a widespread public health problem, being the cause of significant mortality worldwide each year. Primary brain tumors are one of the subgroups of central nervous system tumors and the mechanism underlying their formation remains unknown. This is due to the highly heterogeneous nature of primary brain tumors; histopathological identification of tumor type and molecular epidemiology studies will be helpful in overcoming these difficulties (27-30).

Worldwide, the incidence of primary brain tumors is higher in males; however, the incidence of meningioma, histopathological types of primary brain tumor, is reported to be higher in women (31). According to the 2017 statistics of the American Cancer Society, 56.5% of all patients with primary brain tumor were men, and 43.5% were women (1). The gender distribution of patients in the present study (male: female, %) was 58:42, and thus was consistent with previously reported incidence. The incidence, mortality and survival rates associated with brain tumors are determined by histopathological tumor type,

age, gender and race. Additional risk factors include hereditary factors, alterations in expression levels of cancer-related genes, exposure to ionizing/nonionizing radiation and industrial chemicals, diet, smoking, alcohol, and occupation (26). In the present study, 120 healthy control subjects and 105 patients with primary brain tumor were compared with respect to smoking status and family history of cancer. The groups did not show significant differences with respect to smoking status (P=0.352) and family history of cancer (P=0.560).

Single nucleotide polymorphisms may be used as a tool for investigating genetic variations and disease susceptibility. A number of polymorphisms in the *SOD2* gene have been documented, with polymorphism in the mitochondrial targeting sequence (rs4880 C/T) has been demonstrated to have functional significance (17). In particular, the T allele of the rs4880 C/T polymorphism in the *SOD2* gene has been reported to decrease the efficiency of SOD2 protein (MnSOD) against oxidative stress (32,33), which is supported by the observation that the T-variant decreases formation of the active MnSOD protein in the mitochondrial matrix (17). The association between *SOD2* Ala16Val polymorphism and cancer has been investigated extensively. The Val allele and Val/Val genotype has been associated with an increased risk of lung (34) and bladder (35) cancers, whereas the Ala allele has been associated with an increased risk of breast (23,36), prostate (37,38) and ovarian (39) cancers. Rosenblum *et al* suggested that the Ala16Val polymorphism affects the secondary structure of the protein and may alter the transfer of the enzyme into the mitochondria and its localization (40). Wang *et al* identified an association between Ala16Val polymorphism and lung cancer in Caucasians, and determined that Val/Val was more common in patients with lung cancer, whereas Ala/Ala was more common in control subjects. Comparisons between carriers of Ala/Ala, Ala/Val and Val/Val revealed that Ala/Val and Val/Val were associated with a significantly higher risk (34). Zejnilovic *et al* investigated the association between Ala16Val polymorphism and lung cancer in a Turkish population, and identified that the frequency of the Val/Val was higher in patients compared with in control subjects (26). Meanwhile, Liu *et al* (41) identified that the TT genotype was associated with a significantly higher risk of oral squamous cell carcinoma (P=0.037), and further analysis on genotype dominance indicated a

significant association between the dominant genotype (TT) and oral squamous cell carcinoma ($P=0.013$). In the current study, the frequency of the CC genotype was 24.2%, of the CT genotype was 45.8%, and of the TT genotype was 30.0% in controls. When considering frequencies in patients (23.8, 45.7 and 30.5%, respectively), there were no significant associations between these genotypes and the incidence of primary brain tumor ($P>0.05$). Similar to Liu *et al* (41), the genotypes were also evaluated in terms of associations of codominant, dominant, recessive and overdominant models with tumor risk. However, a significant association between the incidence of primary brain tumor and these genotypes was not determined ($P>0.05$).

Histopathological classification of primary brain tumors demonstrated that astrocytoma was the most common tumor (41.0%). Meningioma was the second most common tumor type (25.7%), and other common tumors included medulloblastoma, colloid cysts, craniopharyngioma, acoustic neuroma (collectively 20.0%) and pituitary adenoma (13.3%). Rajaraman *et al* (12) reported that glioma (astrocytoma) had the highest incidence (64.1%; $n=362$), followed by meningioma (23.7% $n=134$) and acoustic neuroma (12.2%; $n=69$) in the United States. The reported frequency of meningioma by Rajaraman *et al* (12) is consistent with the present study, while the previously observed frequency of astrocytoma is higher. Similar to the present study, Rajaraman *et al* (12) identified no significant association between SOD2 Ala16Val polymorphism and primary brain tumors ($P>0.05$). Contrary to these findings, Zhao *et al* (42) determined a significant association between SOD2 Ala16Val polymorphism and glioma ($P<0.05$). The association between SOD2 Ala16Val polymorphism and primary brain tumors requires further investigation using a larger number of cases and controls.

In conclusion, the present data suggested that SOD2 Ala16Val polymorphism was not a risk factor for primary brain tumor in a Turkish population. To the best of our knowledge, the current study is the first on a Turkish population, and the second on SOD2 Ala16Val polymorphism and primary brain tumor association following investigation by Rajaraman *et al* (12). Further research on polymorphisms associated with SOD2 isoforms (SOD1, SOD3) and other antioxidant enzymes in a larger study population is now required to achieve more comprehensive and accurate data.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

MG diagnosed and treated the patients. AT and YS conducted laboratory test. AT and HP collected the data and analyzed the results. AT and YS wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by local Sivas Cumhuriyet University Ethics Committee (approval no. 2003/304; Sivas, Turkey).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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