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AXIN2 polymorphism and its association with astrocytoma in a Turkish population

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Abstract. The product of *AXIN2*, a component of Wnt signalling, plays a role in tumorigenesis and is dysregulated in cancer cells. In order to determine whether the *AXIN2* polymorphism is a risk factor for astrocytoma, we analysed eight polymorphic regions of this gene in 100 astrocytoma patients compared to 100 healthy controls in a Turkish population using PCR-RFLP methods. For the Exon1-148 T/C, Exon1-432 C/T, Exon5-1365 G/A, Intron5-1712+19G/T, Exon7-2062 C/T and Intron7-2141+73 G/A SNPs of *AXIN2*, no significant association between controls and astrocytoma patients was found. For the Exon5-1386 C/T SNP, a statistically significant association between controls and patients was found ($p<0.05$). For this astrocytoma, patients with the TT genotype showed an increased risk with an OR of 2.92 (adjusted for age, gender and smoking status) (95% CI 1.14-7.47) as compared to the controls with the CC genotype. Our results suggest that *AXIN2* SNPs may be associated with astrocytoma.

Introduction

Although brain tumors account for a small proportion of all cancers (1.4%), most are fatal, and even benign tumors interfere with brain functions essential for daily living (1). Astrocytomas are the most common type of primary human brain neoplasm, accounting for more than 60% of the total. They form a heterogeneous group of tumors and are classified into grades I, II, III and IV (1). The etiology of brain tumors is poorly characterized, and the relationship between heritable and environmental conditions is unclear. The occurence of brain tumors in hereditary syndromes indicates a genetic susceptibility to brain tumors (1). The role of genetic polymorphisms has been widely investigated in many tumor types, and the link between tumor suppressor gene polymorphisms and cancer susceptibility has been reported in the literature (2).

The AXIN homologue *Conductin*, also known as AXIL or AXIN2, functions as a tumor suppressor gene, regulates axis formation during embryonic development and has critical roles in at least three pathways, including the Wnt, stress-activated protein kinase and transforming growth factor β (TGF-β) pathways (3,4). AXIN2 functions as a scaffold protein by approximating all the components required for the phosphorilation of β-catenin, and negatively regulates the Wnt/β-catenin pathway (5,6).

In recent years, many studies on the components of the Wnt pathway have revealed not only the importance of this pathway in normal embryonic development, but also in cancer development. Mutations of Wnt pathway components are associated with numerous human cancers, including colon cancer (7,8), melanoma (9), medullablastoma (10,11), hepatocellular carcinoma (12) and ovarian and uterine cancer (13-15). Polymorphisms and mutations of the *AXIN2* gene have also been implicated in several types of cancer, but have not been investigated in brain tumors (1,3).

Because of its importance in Wnt signalling and carcinogenesis, we analyzed eight polymorphic regions of the *AXIN2* gene in astrocytoma patients in a Turkish population, in order to clarify their association and contribution to this type of tumor.

Materials and methods

Study population. A total of 200 Turkish individuals were studied, including 100 astrocytoma patients who were admitted to the Neurosurgery Department at Cumhuriyet University Hospital in Sivas (central Anatolia) between the years 2006 and 2008. Only patients with newly diagnosed astrocytomas and no previous radiotherapy or chemotherapy were included in the study. There were no gender, age or grade restrictions. All cases and controls were born in Turkey of native Turkish parents. The diagnosis of astrocytoma was histologically confirmed. Age- and gender-matched controls were recruited mainly from patients (with no previous history of cancer or of radio- or chemotherapy) at the same hospital (n=100). All subjects gave their informed consent for participation in the study and completed a short questionnaire, including questions about occupation, tobacco use, alcohol consumption and history of cancer. The local university ethics committee on human research approved the study.

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Table I. Characteristics of astrocytoma patients and healthy controls.

	Patients (n=100) (%)	Controls (n=100) (%)	p-value
Gender			0.089
Male	58 (58)	46 (46)	
Female	42 (42)	54 (54)	
Age (years ± SD)	58.66±8.039	57.01±7.89	
Smoking habit			0.181
Non-smoker	61 (61)	70 (70)	
Smoker	39 (39)	30 (30)	
Alcohol consumption			0.052
No	92 (92)	98 (98)	
Yes	8 (8)	2 (2)	
Family history of cancer			0.060
No	89 (89)	96 (96)	
Yes	11 (11)	4 (4)	

p-values were calculated by the χ^2 test.

DNA analysis. Blood samples from patients and controls were collected in citrate-containing tubes. DNA was extracted from blood by a salting out procedure and stored at -20˚C until analysis (16).

A total of eight regions, including 5 exons and 3 introns of the *AXIN2* gene, were amplified by PCR in 30 cycles using previously reported primers (17). PCR was performed in a reaction volume of 25 μl, containing 100 ng of genomic DNA, 10 pmol of the appropriate amplification primers, 5 nmol each of four deoxynucleotide triphosphates (Fermentas), 2.5 units of *Taq* DNA polymerase (Fermentas), 10 mmol/l Tris-HCl (pH 8.3 at 25˚C), 50 mmol/l KCl and 1.5 mmol/l $MgCl_2$. PCR conditions consisted of an initial denaturation at 94˚C for 5 min, followed by 30 cycles at 94˚C for 30 sec, 60˚C for 30 sec and 72˚C for 60 sec, followed by 1 cycle at 72˚C for 5 min.

In order to detect the Exon1-148 C/T, Exon1-432 T/C, Intron2-956+16 A/G, Exon5-1365 G/A, Exon5-1386 C/T, Intron5-1712+19G/T, Exon7-2062 C/T and Intron7-2141+73 G/A SNPs of the *AXIN2* gene, PCR products were digested with 5 units of *Mph1103* I, *Esp3* I, *Mls* I, *Mps* I, *Taq* I, *Cpo* I, *Taq* I and *BseR* I restriction endonucleas enzymes, respectively (Fermentas) in a total reaction volume of 10 μl containing 1X reaction buffer (supplied with the enzyme) for 4 h at the optimum temperature according to the manufacturer's instructions.

Amplified and digested DNA fragment sizes were as previously reported (17). Following digestion, DNA fragments were seperated on 2.5% agarose gels.

Statistical analysis. Statistical analysis was performed using the Statistical Package for Social Sciences program (SPSS, version 16). The χ^2 test was used to evaluate the association between astrocytoma incidence, tobacco use and *AXIN2* genotypes. Statistically significant departures from Hardy-Weinberg equilibrium for the controls were assesed using the χ^2 test.

Results

Characteristics of primary brain tumor patients and healthy controls. A total of 200 individuals (100 patients with astrocytomas and 100 healthy controls) were genotyped for eight regions of the AXIN2 gene. The main charactersitics of the study population are presented in Table I. The mean ages of the patients and controls were 58.66±8.039 and 57.01±7.89 years, respectively. The frequency of males and females was 58 and 42% among the astrocytoma cases and 46 and 54% among the controls, respectively. Although a family history of cancer was more prevalent in the cases (11%) than in the controls (4%), no statistically significant association was found. The proportion of smokers was significantly higher among patients (39%) than controls (30%), and in both groups the majority of smokers were male.

Statistical analysis. Eight regions of the *AXIN2* genotype in patients and controls, including allele frequency, genotype distributions, p-values and adjusted OR and 95% CI values, are presented in Table II. The groups were in Hardy Weinberg equilibrium.

For the Exon1-148 T/C, Exon1-432 C/T, Intron2-956+16 A/G, Exon5-1365 G/A, Intron5-1712+19G/T, Exon7-2062 C/T and Intron7-2141+73 G/A SNPs of the AXIN2 gene, no significant association was found between controls and astrocytoma patients.

For the Exon5-1386 C/T SNP, the frequency of the CC, CT and TT genotypes was 42, 50 and 8% in the controls, and 36, 44 and 20% in patients, respectively, indicating a statistically significant association between controls and patients. When compared to controls with the CC genotype, patients with the TT genotype showed an increased risk, with an OR of 2.92 (adjusted for age, gender and smoking status) and 95% CI 1.14-7.47.

Table II. *AXIN2* genotypes in healthy controls and astrocytoma patients.

AXIN2 genotype	Patients (%)	Controls (%)	p-value	OR (95% CI)	
				Crude	Adjusted[a]
Exon1-148 C/T					
CC	39 (39)	32 (32)	1	1 (Reference)	1 (Reference)
CT	45 (45)	52 (52)	0.274	0.71 (0.38-1.31)	0.68 (0.36-1.28)
TT	16 (16)	16 (16)	0.643	0.82 (0.35-1.89)	0.61 (0.25-1.51)
Allele frequencies					
C	123 (61.5)	116 (58)			
T	77 (38.5)	84 (42)			
Exon1-432 T/C					
TT	93 (93)	95 (95)	1	1 (Reference)	1 (Reference)
TC	7 (7)	5 (5)	0.352	1.80 (0.51-6.37)	1.84 (0.51-6.57)
CC	0	0			
Allele frequencies					
T	193 (96.5)	195 (97.5)			
C	7 (3.5)	5 (2.5)			
Intron2-956+16 A/G					
AA	70 (70)	64 (64)	1	1 (Reference)	1 (Reference)
AG	25 (25)	28 (28)	0.532	0.81 (0.43-1.54)	0.81 (0.41-1.56)
GG	5 (5)	8 (8)	0.343	0.57 (0.17-1.83)	0.62 (0.19-2.02)
Allele frequencies					
A	165 (82.5)	156 (78)			
G	35 (17.5)	44 (22)			
Exon5-1365 G/A					
GG	91 (91)	88 (88)	1	1 (Reference)	1 (Reference)
GA	9 (9)	12 (12)	0.489	0.72 (0.29-1.80)	0.73 (0.29-1.86)
Allele frequencies					
G	191 (95.5)	188 (94)			
A	9 (4.5)	12 (6)			
Exon5-1386 C/T					
CC	36 (36)	42 (42)	1	1 (Reference)	1 (Reference)
CT	44 (44)	50 (50)	0.932	1.02 (0.56-1.87)	0.97 (0.52-1.79)
TT	20 (20)	8 (8)	0.022	2.91 (0.14-7.41)	2.92 (1.14-7.47)
Allele frequencies					
C	116 (58)	134 (67)			
T	84 (42)	66 (33)			
Intron5-1712+19G/T					
TT	91 (91)	96 (96)	1	1 (Reference)	1 (Reference)
GT	9 (9)	4 (4)	0.152	2.37 (0.70-7.97)	2.00 (0.58-6.93)
Allele frequencies					
T	191 (95.5)	196 (98)			
G	9 (4.5)	4 (2)			
Exon7-2062 C/T					
CC	87 (87)	86 (86)	1	1 (Reference)	1 (Reference)
CT	13 (13)	14 (14)	0.836	0.91 (0.40-2.06)	0.86 (0.37-1.96)
Allele frequencies					
C	187 (93.5)	186 (93)			
T	13 (6.5)	14 (7)			
Intron7-2141+73 G/A					
GG	82 (82)	80 (80)	1	1 (Reference)	1 (Reference)

Table II. Continued.

AXIN2 genotype	Patients (%)	Controls (%)	p-value	OR (95% CI)	
				Crude	Adjusted[a]
GA	18 (18)	20 (20)	0.718	0.87 (0.43-1.78)	0.80 (0.38-1.65)
Allele frequencies					
G	182 (91)	180 (90)			
A	18 (9)	20 (10)			

p-values were calculated by the χ^2 test. [a]Adjusted for age, gender and smoking status.

Discussion

Primary brain tumors represent a very heterogenous group of diseases that are poorly understood. Among neurocarcinogenic factors, chemical, physical and genetic factors play important roles. Specific genetic polymorphisms, most commonly those involved in DNA repair, carcinogen metabolism and immune function genes, have been studied in brain tumors, and some promising results have been reported (18-20). The association between carcinogenesis and the *AXIN2* polymorpism was recently investigated in a few populations (21-23). The multidomain scaffold protein *AXIN2*, which is also a tumor suppressor, plays important roles in various biological processes, including Wnt and TGF-β signalling (24-27). For the degradation of β-catenin, it is essential that a component of Wnt signalling be frequently dysregulated in cancer cells (28,29). Thus, the genetic variations that affect the *AXIN2* gene may be susceptibility factors for brain tumors. Here, we studied eight polymorphic regions of the *A*XIN2 gene in astrocytomas, the most common type of primary human brain tumor, in a Turkish population.

In this study, the *AXIN2* polymorphism was investigated in 100 astrocytoma patients in a Turkish population, and the results were compared to those from 100 hospital-based controls. Although the frequency of males (58%), smokers (39%), family history of cancer (11%) and alcohol consumption (8%) was higher among patients than among controls, we found no significant association between the above factors and the studied *AXIN2* SNPs.

Mutation studies of the *AXIN* genes in different types of cancer are well documented (30-33). It has also been reported that *AXIN2* SNPs are associated with lung and breast cancers, but not with colorectal and head and neck cancers (21,23).

In our study, we found a significant relationship between the TT genotype of the *AXIN2* Exon5-1386 C/T region and astrocytoma risk. The frequency of the TT genotype in patients was higher (20%) than in controls (8%), which suggests that it is risk factor for astrocytoma development. This finding was confirmed statistically; patients with the TT genotype showed an increased risk of cancer with an adjusted OR of 2.92 (95% CI 1.14-7.47). Although this region is important for β-catenin binding on scaffold protein, this SNP has no effect on the protein (3). Insertion and deletion mutations of *AXIN2* have been reported in Exon5 (22). It is possible that this SNP together with other mutations contributes to the development or progression of astrocytoma.

We found no association between the incidence of astrocytomas and seven regions of the *A*XIN2 gene, including Exon1-148 T/C, Exon1-432 C/T, Intron2-956+16 A/G, Exon5-1365 G/A, Intron5-1712+19G/T, Exon7-2062 C/T and Intron7-2141+73 G/A. Although Kanzaki *et al* and Gunes *et al* reported a significant association between lung cancer and the *AXIN2* Exon1-148 C/T polymorphism causing a Pro50Ser change on the protein in a Japanese population and a Turkish population (21,23), we did not observe any association in our study population, even though the genotype distribution of our control group for this region of *AXIN2* was similar to that of the Japanese population.

Another notable finding of the present study was that, although an SNP at the Exon5-1365 G/A region of the *A*XIN2 gene has been reported to be an A/G transition (dbSNP ID: rs9915936), no AA genotype was identified in our control or study groups (34). Therefore, the GG genotype was taken to be the wild-type genotype in our statistical tests.

Gao *et al* investigated the association of *AXIN2* SNPs in Hirschsprung (HSCR) disease, and found that the Exon2-rs2240308 and Exon6-rs9913621 polymorphisms contribute to HSCR susceptibility, while Exon5-rs8081536 SNP had no effects on this disease (35).

In another study, the association of genetic variation in genes implicated in the β-catenin destruction complex with breast cancer risk was reported (22). Five SNPs of *AXIN2* and one SNP of APC were found to be associated with an increased risk of breast cancer, suggesting that this pathway plays a role in carcinogenesis. It seems that SNPs of *AXIN2* contribute to different biological processes, in particular to tumorigenesis. Our findings support this hypothesis.

In conclusion, this is the first study carried out in a Turkish population investigating the *AXIN2* polymorphism and its association with astrocytomas. Our results suggest that the risk of primary brain tumors may be related to the *AXIN2* polymorphism, at least in our study population. Further investigations in different populations with large sample numbers are required to support our hypothesis. We believe that the accumulation of knowledge about the genetic changes that make a person susceptible or resistant to brain tumors may eventually result in the prevention of the formation of this disease.

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