Deoxy-ribonucleic acid repair genes *XRCC1* and *XPD* polymorphisms and brain tumor risk

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ABSTRACT

الأهداف: تقييم ما إذا كان لتكوين المتعدد لصبغيات (XRCC1) و(XPD)لإصلاح الحمض النووي (DNA) فعالية في أورام الدماغ.

الطريقة: أجرينا دراسة مبنية على حالة السكان شملت 135 حالة مصابة بأورام الدماغ و87 شخصاً سليماً بناء على العمر، ونوع الجنس، يمثلون مجموعة الأصحاء (مجموعة التحكم) تم فحص دور التكوين المتعدد لصبغيات الجين (XRCC1 Arg399Gln –) وجين (XPD– Lys75lGln) في مخاطر تكوين ورم الدماغ للسكان الأتراك في الفترة ما بين عام 2004م وحتى2007م، بجامعة سيلكوك – تركيا. تم تقسيم المرضى المصابين بأورام الدماغ إلى مجموعات فرعية، الأورام الدبقية (عدد=71)، الورم السحائي (عدد=35)، الورم الغدي النخامي (عدد= 21)، والانتشار إلى الدماغ (عدد=8). تم تحليل تشخيص أورام الدماغ في جميع المرضى بواسطة الفحص النسيجي المرضي. تم عزل الحمض النووي الصبغي (GDNA) لتحليل الخلايا البيضاء بتفاعل سلسلة الخمائر الناقلة.

النتائج: كان اتحاد نوع الجين لكلاً من (– XRCC1 Arg399Gln) و(XPD – Lys75lGln) مع نوع الورم. كانت الأورام وفقاً لنوع الدماغ الفرعي كالتالي 71 (52.6%) الورم السحائي ، 35 الورم الدبقي (25.9%)، 21 (15.55%)، الورم الغدي نخامي، و8 (5.9%) انتشار الورم في الدماغ. أما بالنسبة للأورام الفرعية كان هنالك فرق ملحوظ في صبغيات (XRCC1 Arg399Gln –) ولكن ليس لدى النوع الجيني (Lys75lGln XPD–) بين النوع الفرعي للأورام.

خاتمة: هذان التكوينان المتعددان في الجينين يشيران إلى عدم وجود عامل خطر مرتفع لأورام الدماغ في الأفراد الذين لديهم عامل خطر لتكوين نوع الجين (XRCC1 – Arg399Gln) و(Lys75lGln XPD–).

Objectives: To evaluate whether polymorphisms in the deoxy-ribonucleic acid (DNA) repair genes *XRCC1* and *XPD*, have efficacy in the development of brain tumors.

Methods: This is a case-population based study, including 135 cases of brain tumors, and 87 population based age- and gender-matched healthy controls. We examined the role of *XRCC1 Arg 399Gln* gene and *XPD Lys751Gln* gene polymorphisms, in the context of brain tumor risk for the Turkish population between 2004 and 2007 at Selcuk University, Konya, Turkey. Patients with brain tumors were subdivided into glial tumors (n=71), meningiomas (n=35), pituitary adenomas (n=21), and metastases to the brain (n=8). The diagnoses of brain tumors in all patients were analyzed by histopathological examination. Genomic DNA of leukocytes for polymerase chain reaction analysis was isolated.

Results: Association of genotype of both *XRCC1 Arg399Gln* and *XPD Lys751Gln* genotypes with tumor types, tumors according to brain subtypes were, 71 (52.6%) meningiomas, 35 glial (25.9%), 21 (15.55%) pituitary adenomas, and 8 (5.9%) metastases to the brain. Between subtypes of tumors, there was a significant difference in *XRCC1 Arg399Gln* genotypes, and not in *XPD Lys751Gln* genotypes.

Conclusion: The results indicated no elevated risk for brain tumors in individuals with the *XRCC1 Arg399Gln* and *XPD Lys751Gln* polymorphism risk.

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Individual susceptibility for tumor development depends on a complex interaction between environmental and genetic factors.[1,2] Several case-control studies have been conducted to carry out human cancer etiology on the basis of environmental and genetic

factors. Epidemiological studies showed a wide range of possible risk factors including diet, smoking, alcohol, occupation, and industry exposure to ionizing or non-ionizing radiation, allergies, infections, family history, and inherited polymorphisms in genes related to carcinogen metabolism, oxidative metabolism, and deoxy-ribonucleic acid (DNA) repair genes.[3] Recent research has evaluated the role of common polymorphisms of selected DNA repair genes, and the susceptibility or risk of human cancers. Therefore, genetic epidemiology has become an important tool for linking human cancers with inherited alterations in genes regulating DNA repair processes, and with specific environmental exposures. Several studies showed that predisposition to many cancers have now been associated with the inheritance of polymorphisms in genes, either single or in combination.[4] In the literature, the polymorphisms of DNA repair *XRCC1* and *XPD* genes have been reported to be associated with the risk of several types of cancer, however, the genetic factors that contribute to brain tumor etiology are poorly understood, and there is only one study on the association of *XRCC1 Arg399Gln* and *XPD Lys751Gln* with brain tumors.[5] The possible association of these polymorphisms with brain tumors susceptibility has not yet been fully evaluated. Therefore, in this hospital-based case-control study, we evaluated the role of *XRCC1 Arg399Gln* and *XPD Lys751Gln* polymorphisms in brain tumors. We aimed at examining the genotype effects in terms of brain tumor etiology, tobacco smoking, which is known to cause DNA damage repaired by DNA repair enzymes of the base-excision repair (BER) and nucleotide-excision repair (NER) pathways. The purpose of this study was to evaluate, whether polymorphisms in the DNA repair genes *XRCC1* and *XPD*, have efficacy in the development of brain tumors.

Methods. This case-control study was carried out at Selcuk University Medical Research and Application Center, and the Department of Neurosurgery, Konya, Turkey. The study group consisted of 135 patients with brain tumors, and 87 healthy individuals as a control group. Ethical approval from the local ethics committee and informed consent was obtained prior to participation in the study. The diagnoses of brain tumors in all the patients were analyzed by histopathological examination. Data for 135 patients at diagnosis were obtained from our hospital records, including age, gender, family history, smoking, alcohol habits, and tumor histopathology type. Patients diagnosed with brain tumors were invited to our clinics and were interviewed, to confirm the history regarding smoking, and alcohol habits. Patients with brain tumors were subdivided into glial tumors (n=71), meningiomas (n=35), pituitary adenomas (n=21), and metastases to the brain (n=8). Tumor histopathological types and clinical stages were classified according to the guidelines of the World Health Organization. Control group subjects were chosen from the same population in the same geographic area. Exclusion criteria for control individuals were malignancy, age >18 years old, and with the presence of any metabolic disease or allergic disease in the patient or family.

Deoxy-ribonucleic acid extraction, polymerase chain reaction, restriction fragment length polymorphism and genotyping of XRCC1 Arg399Gln and XPD Lys751Gln. Genomic DNA of leukocytes from patient and control individuals for polymerase chain reaction (PCR) analysis was isolated using a commercial DNA isolation kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The *XRCC1 Arg399Gln* and *XPD Lys751Gln* genotypes were identified by PCR-RFLP using a set of primers to amplify a 615 base pair (bp) sequence of the *XRCC1 Arg399Gln* gene 6, and a 235 bp sequence of the *XPD Lys751Gln*.[6] To detect the genotypes of *XRCC1 Arg399Gln* and *XPD Lys751Gln*, 2 pair primers were used, 5'- TTG TGC TTT CTC TGT GTC CA-3' and 5'-TCC TCC AGC CTT TTC TGA TA-3' for *XRCC1,* and 5'-CCT CTG TTC TCTGCA GGA GGA-3' and 5'-CCT GCG ATT AAA GGC TGT GGA -3' for *XPD*. The PCR reactions were conducted in a thermal cycler (GeneAmp 2700, PerkinElmer-ABI, Foster City, CA). The 20 µl reaction mixture contained 4 µl of genomic DNA, 10 x PCR buffer (50 mmol/L potassium chloride, 50 mmol/L Tris-chloride, and 1.25 mmol/L magnesium chloride), 5 mmol/L deoxynucleotide triphosphate mixtures, 10 mmol/L of each primer, 0.5 U of Taq DNA polymerase (Vivantis, Indonesia), and sterile distilled water. After a 3 minute incubation at 94°C, a first round of 10 cycles was programmed (15 seconds (sec) at 94°C, 15 sec at 59°C, and 15 sec at 72°C), followed by a second round of 25 cycles (15 sec at 94°C, 15 sec at 57°C, and 15 sec at 72°C, with a 3-second/cycle increase in extension time). In order to check the presence of the PCR products, 5 µl of the product was added to 1% agarose gel. The PCR products (615 bp for *XRCC1 Arg399Gln*, 235 bp for *XPD Lys751Gln* were resolved on electrophoresis with ethidium bromide-stained 1% agarose gel, and were sized (shaped) using a DNA molecular weight marker under ultraviolet light. The remaining PCR products were digested by using restriction enzymes MspI for *XRCC1 Arg399Gln* and PstI enzymes for *XPD Lys751Gln*, (Ferment's International, Burlington, Canada). The products were resolved on 2% agarose gel.

Statistical evaluation. The statistical evaluation of results was performed in SPSS for Windows software package (SPSS, Chicago, IL). The associations between

Table 1 - Distribution of *XRCC1* and *XPD*-repair gene genotype and allele frequency.

Groups	Genotype of *XRCC1 (Arg399Gln)*			Allele frequency of *XRCC1*	
	Arg/Arg	*Arg/Gln*	*Gln/Gln*	*Arg*	*Gln*
Patient (%)	(37.8)	(54.1)	(8.1)	(64.85)	(35.15)
Control (%)	(49.4)	(47.1)	(3.4)	(72.95)	(26.95)
	x^2=4.04, *p*=0.13			x^2=2.66, *p*=0.26	
	Genotype of *XPD (Lys751Gln)*			Allele frequency of *XPD*	
	Lys/Lys	*Lys/Gln*	*Gln/Gln*	*Lys*	*Gln*
Patient (%)	(40)	(50)	(3)	(65)	(28)
Control (%)	(42.5)	(57.5)	(0)	(71.25)	(28.75)
	x^2=5.59, *p*=0.23			x^2=0.89, *p*=0.34	

Arg - Arginine, Gln - glutamine, Lys - lysine

Table 2 - Clinical and histopathological characteristics among *XRCC1* and *XPD* phenotypes.

Characteristics	*XRCC1 (Arg399Gln)* genotype			*XPD(Lys751Gln)* genotype			*XRCC1* and *XPD* combination	
	Arg/Arg	*Arg/Gln*	*Lys/Lys*	*Lys/Gln*	*Gln/Gln*	*Gln/Gln*	*(Arg/Gln) + (Gln/Gln)*	*(Lys/Gln) + (Gln/Gln)*
	%							
Association with gender								
Men (n=68)	44.5	48.2	7.3	41.8	55.5	2.7	67.3	32.7
Women (n=67)	40.2	54.5	5.4	40.2	58.9	0.9	67	33
	x^2=0.99, df=2, *p*=0.60			x^2=1.19 , df=2, *p*=0.55			OR=1.01, (95% CI:0.57-1.77)	
Association with age								
≤55 (n=81)	42	51.9	6.2	44.4	54.3	1.2	69.1	30.9
>55(n=54)	43.3	50	6.7	31.7	65	3.3	61.7	38.3
	x^2=0.06, df=2, *p*=0.96			x^2=3.68, df=2, *p*=0.15			OR=1.39, (95% CI:0.75-2.58)	
Association with tobacco smoking								
Smoker (n=32)	46.9	40.6	12.5	37.5	59.4	3.1	65.6	34.4
Nonsmoker (n=103)	35	58.3	6.8	40.8	56.3	2.9	64.1	35.9
	x^2=3.29, df=2, *p*=0.19			x^2=0.10, df=2, *p*=0.94			OR=1.07, (95% CI:0.46-2.46)	
Association with tumor type								
Meningiomas (n=71)	35.2	57.7	7	46.5	50.7	2.8	67.6	32.4
Glial tumors (n=35)	57.1	37.1	5.7	25.7	71.4	2.9	71.4	28.6
Pituitary adenomas (n=21)	28.6	52.4	19	38.1	61.9	0	47.6	52.4
Metastases (n=8)	0	100	0	50	37.5	12.5	50	50
	x^2=16.33, df=6, *p*=0.01			x^2=8.22, df=6, *p*=0.22			x^2=4.37, df=3, *p*=0.22	
Association with tumor type (not including metastasis group)								
Meningiomas (n=71)	35.2	57.7	7	46.5	50.7	2.8	67.6	32.4
Glial tumors (n=35)	57.1	37.1	5.7	25.7	71.4	2.9	71.4	28.6
Pituitary adenomas (n=21)	28.6	52.4	19	38.1	61.9	0	47.6	52.4
	x^2=8.78, df=4, *p*=0.67			x^2=4.95, df=4, *p*=0.29			x^2=3.64, df=2, *p*=0.16	

OR - odds ratio, CI - confidence interval, df - degrees of freedom, Arg - Arginine, Lys - lysine, Gln - glutamine

the genotype frequencies of *XRCC1 Arg399Gln* and *XPD Lys751Gln*, and the control and patient groups were assessed using odds ratio, and confidence intervals (95% CI). The *XRCC1 Arg399Gln* and *XPD Lys751Gln* genotype distributions were compared between groups using a x^2 test.

Results. The distribution of *XRCC1 Arg399Gln* and *XPD Lys751Gln* genotypes, and allele frequency in the control and patient groups are shown in **Table 1**, as identified from PCR-RFLP analyses. There was no significant difference in *XRCC1 Arg399Gln* and *XPD Lys751Gln* genotypes and allele frequencies between study and control group (**Table 1**).

Association of XRCC1 Arg399Gln and XPD Lys751Gln genotypes with age, gender, tobacco smoking and alcohol consumption. The patients with brain tumors (ranged 6-80 years old, average 55.2 ± 7.63, median: 55 years, 68 males and 67 females), were divided into 2 age groups relative to the median age: younger, ≤55 years old (n=81), and older, >55 years old (n=54). The mean age in patients with brain tumors were slightly higher than control healthy individuals, however, the difference were not significant (p>0.05). There was also no significant difference in the distribution of *XRCC1 Arg399Gln* and *XPD Lys751Gln* genotypes between the young patient and old patient groups and between male and female patients (**Table 2**). The patients were divided into 2 groups as smoker and non-smoker. Out of the 135 patients, 32 (23.7%) had a history of tobacco smoking. There was no significant difference in distribution of *XRCC1 Arg399Gln* and *XPD Lys751Gln* genotypes between smoker and non-smoker patients (**Table 2**). Only 2 patients were alcohol consumers.

Association of XRCC1 Arg399Gln and XPD Lys751Gln genotypes with tumor type. Association of genotype of both *XRCC1 Arg399Gln* and *XPD Lys751Gln* genotypes with tumor type tumors according to brain subtypes were, 71 (52.6%) meningiomas, 35 glial (25.9%), 21 (15.55%) pituitary adenomas, and 8 (5.9%) metastases to the brain. Between subtypes of tumors, there was a significant difference in *XRCC1 Arg399Gln* genotypes, and this significance was due to metastases to the brain. However, there was not a significant difference in *XPD Lys751Gln* genotypes between the subtype of tumors (**Table 2**). As each subtype of tumor was compared with the control group, there was also a significant difference in *XRCC1 Arg399Gln* genotypes only in pituitary adenomas (x^2=8.07, p=0.01) and metastases subgroups (x^2=8.20, p=0.01). In subgroups of meningiomas (x^2=1.16, p=0.55), and glial tumors (x^2=3.68, p=0.15) there was no significant difference as compared with control groups. For *XPD Lys751Gln* genotypes, there was no significant difference between control and tumor subtypes (p>0.05).

Discussion. In this study, we examined the DNA repair genes *XRCC1* and *XPD*, involved in BER and NER repair systems, as candidate susceptibility genes for brain tumors in a hospital based case-control study of the middle of Anatolia, Turkey. In our population, there was no significant difference in the allele frequency of the *XRCC1 Arg399Gln* and *XPD Lys751Gln* genes, between control and patient groups. However, there was significant difference in the allele frequency for *XRCC1* gene in different population in the literature reviewed by Erdal et al.[7] Our results are almost similar to the allele frequencies of *XRCC1* and *XPD* reported by Canalle et al,[8] and de las Penas et al.[9] In contrast, in 2 previous reports regarding healthy individuals in the different geographic regions of our population, the allelic frequency of *XRCC1 399Arg* was stated as 60%[7] and 65%,[10] which are lower than in our control (74%), however, similar to the patient group (64%). As for *XPD Lys751Gln* genes in the present study, there were no significant differences in allelic frequency between patient and control groups. However, in a previous report related to healthy individuals, the allelic frequency of *XPD Lys751* was stated as 51%,[11] which is lower than in our control (71%), and patient groups (65%). These results indicate that the allele frequency of *XRCC1Arg399Gln* polymorphism of genes vary from population to population even from geographic region to region. Similar differences were reported in the literature reviewed by Erdal et al.[7]

One of the most common and functional *XRCC1* polymorphisms is *Arg399Gln*. Its function has not been elucidated in many cancers, however, this polymorphism may be associated with a reduced repair capacity, and increased susceptibility to some cancers. Previous studies have suggested that the *XRCC1 399Gln* polymorphism is associated with increased levels of DNA damage in human cells exposed to various mutagens,[12-14] with increased risk of stomach,[13] head and neck,[14] and lung cancers,[15] however, others have reported that the *399Gln* polymorphism has no adverse effect on DNA repair.[16,17] In relation to brain tumors, there was only one report related to *XRCC1 Arg399Gln* polymorphism in the literature. Wang et al[5] reported no association between distribution of *XRCC1 399 Arg/Gln* polymorphism, and glial tumors when compared to control group. In this present study, we did not find any significant differences of distribution of *XRCC1 Arg399Gln* polymorphism between patients and controls, and also patient demographic data such as gender, age, tobacco smoking, tumor and tumors subtypes, however, there was a significant relation between *XRCC1 Arg399Gln*

polymorphism and pituitary tumors and metastases. The limitation of this study was that the size of the subgroup, metastasis to brain, was not large as some patients could not be included as their primary cancer had caused their death. Therefore, it needs further study to clarify the association between those in large populations. There has been no report in the literature regarding the association of *XRCC1 Arg399Gln* polymorphism with meningiomas and pituitary tumors. Many polymorphisms in the *XPD* gene have also been identified, and the most common, and important functional polymorphism at exon 23 is *XPD Lys751Gln* polymorphism. This polymorphism is associated with lower DNA repair capacity.[18] Therefore, several studies have investigated the association between *XPD Lys751Gln* polymorphism and cancer. Some of these studies have reported significant associations between *Lys751Gln* polymorphism and predisposition to bladder cancer,[19] prostate cancer,[20] melanoma,[21] breast cancer,[22] and lung cancer,[23] and some studies did not find any association between this polymorphism and cancers. Metsola et al[24] and Forsti et al[25] reported that there was no significant association between breast cancer and the *XPD Lys751Gln* genotypes. Mort et al,[26] and Yeh et al[27] also reported no significant association between the *XPD Lys751Gln* polymorphism and colorectal cancer. For brain tumors, in the literature there is only one report related to the brain tumor such as glial tumors, although they did not find any association between distributions of *XPD Lys751Gln* and glial tumors.[5] In the present study, we did not find any significant association between distributions of *XPD Lys751Gln* polymorphism and patients' age, gender, tobacco smoking, and tumor histopathology subtypes, indicating no functional role in the development of brain cancer and a prognostic significance in patients with brain tumors.

The phenotypic effects of an individual polymorphism may be obscured, as DNA repair mechanisms include a number of factors and mechanisms through the repair pathways. Interaction between various gene products in the additive or synergistic effects may increase cancer risk. In the present study, we evaluated the effects of combining the homozygous and heterozygous polymorphism of the *XRCC1 Arg399Gln* and *XPD Lys751Gln* genes as regard to brain tumor risk. Our results showed that when polymorphisms from *XRCC1 Arg399Gln* and *XPD Lys751Gln* get together, they eliminate brain tumor risk regardless of gender, age, tobacco smoking factors and tumor histopathologic subtypes (**Table 2**).

In conclusion, although the sample sizes of the subgroups of patients with brain tumors were not sufficiently large to detect any true effects of polymorphisms on brain tumors risk, this study evaluated the possible association between the *XRCC1 Arg399Gln* and *XPD Lys751Gln* polymorphism, and brain tumor risk. Our results indicated no elevated risk for brain tumors in individuals with the *XRCC1 Arg399Gln* and *XPD Lys751Gln* polymorphism risk. These results need to be confirmed in larger patient subgroups, such as meningiomas, glial tumors, pituitary adenomas, and metastasis, to fully understand the effects of these polymorphisms.

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ETHICAL CONSENT

All manuscripts reporting the results of experimental investigations involving human subjects should include a statement confirming that informed consent was obtained from each subject or subject's guardian, after receiving approval of the experimental protocol by a local human ethics committee, or institutional review board. When reporting experiments on animals, authors should indicate whether the institutional and national guide for the care and use of laboratory animals was followed. Research papers not involving human or animal studies should also include a statement that approval/no objection for the study protocol was obtained from the institutional review board, or research ethics committee.