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Functional variants of *p21* gene alter susceptibility to meningioma

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Meningioma is the most common brain tumour, arising from arachnoid cells of the meninges. It accounts for almost 37% of all primary brain tumours. According to the World Health Organization (WHO), meningioma is categorized into three grades. The recurrence rate of grade I, II and III at 5 years after complete resection is approximately 10, 50 and 80%, respectively [1]. The complex aetiology of meningioma involves a combination of environmental factors and genetic impairments. Head trauma, occupational exposure, mobile phones, female sex hormones and smoking may contribute to meningioma [2]. *p21* (also named CDKN1A or WAF1/Cip1) is a central mediator of the p53 checkpoint control on chromosome 6p21.2. *p21* protein has multiple functions, such as control of cell motility, regulation of apoptosis, transcriptional activity, inhibition of cell proliferation and action as a tumour suppressor [3].

Genetic variations in *p21* may lead to a change of gene expression and induce human tumourigenesis. One of the most prominent functional polymorphisms identified within the coding region of *p21* is C98A (dbSNP rs1801270) at codon 31. At this site, a substitution of C to A in the third base of codon 31, results in a Serine to Arginine substitution in DNA-binding zinc finger motif of *p21* protein [4]. Another *p21* polymorphism (dbSNP rs1059234) is located at nucleotide position 70 (C to T) within the 3′ untranslated region, downstream of exon 3 stop codon [5]. This site may alter expression, as this region of the mRNA interacts with various non-coding RNAs and proteins that regulate mRNA stability and translation. To date, several studies have evaluated the role of *p21* C98A and C70T polymorphisms in diseases such as prostate cancer, cervical neoplasia and Kaposi's sarcoma [6–8]. The association between *p21* C98A polymorphism and meningioma risk has not been reported. We conducted a case-control study to determine the association between *p21* C98A and C70T polymorphisms and the risk of meningioma.

We recruited 225 patients with meningioma and 320 healthy volunteers. Meningioma cases were confirmed by magnetic resonance imaging and histopathology according to the WHO classification. Patients who had any history of cancer/intracranial surgery, and received either radiotherapy or chemotherapy before surgery were excluded. The physical examination and imaging results of the patients at presentation and follow-up were recorded. We subsequently evaluated recurrence using radiological imaging at follow-up. Controls with a self-reported history of central nervous system-related diseases or cancer and previously receiving radiotherapy/chemotherapy were excluded. The research protocol was performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Guilan University of Medical Sciences. Informed consent was obtained from all subjects.

One ml of blood was collected before surgery. Genomic DNA was extracted from peripheral blood cells using the GPP Solution (Gen Pajoohan, Tehran, Iran) according to manufacturer's instruction. Purity and quantity of DNA was checked with a spectrophotometer with A260/280 1.75–1.85. Extracted DNA was stored at −80 °C until use. To identify *p21* C98A and C70T SNPs, genotyping was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Target fragments of the *p21* gene were amplified with specific primers according to previous reports [9, 10]. Primers were synthesized by MWG-Biotech (Ebersberg, Germany). Each PCR mixture consists of 25 µl comprising 1X PCR buffer, 1.5 mM $MgCl_2$, 0.5 µM of both forward and reverse primer, 200 µM dNTPs and 2U Taq polymerase (Gene Fanavaran, Tehran, Iran) and 200 ng of DNA. The temperature profiles were as follows, for *p21* C98A variation: Initial denaturation step of 95 °C for 5 min followed by

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35 cycles at 95 °C for 30 s, 63.7 °C for 30 s, 72 °C for 40 s, and a final 5-min extension step at 72 °C. The reaction conditions for *p21* C70T were the same as described for *p21* C98A variation (except annealing at 65 °C for 30 s). For the determination of *p21* C98A and C70T variations, PCR products were digested with *BlpUI* and *PstI* (New England BioLabs, Hitchin, UK) according to the manufacturer's recommendations with electrophoresis on 2.5% agarose gels, respectively. Genotypes were determined as AA (homozygous mutant genotype) (220 bp), CC (wild type genotype) (127, 93 bp) or CA (220, 127, 93 bp) for *p21* C98A variation and CC (173, 125 bp), TT (298 bp) or CT (298, 173, 125 bp) for *p21* C70T.

The OpenEpi software (www.OpenEpi.com) based on minor allele frequency (MAF) of SNPs was used to calculate a required sample size. Allele and genotypes frequency differences of each SNP between cases and controls were evaluated using the Chi-square test and assessed as an odds ratio (OR) of the minor allele with 95% confidence interval (CI) and *p*-value, using the major allele as a reference. The goodness of fit χ^2 test was used to assess deviations from the Hardy–Weinberg equilibrium (HWE). Associations between alleles/genotypes and meningioma occurrence were considered significant at $p<0.05$.

Patients with Grade II and Grade III meningioma were 153 (68%) female, 72 (32%) male with the average age of 38.6 years. The control group were 62.5% female and 37.5% male with the average age of 36.5 years. There were no significant differences in age and sex between cases and controls ($p = 0.69$ and $p = 0.18$, respectively). Genotype frequencies of the SNPs did not deviate from HWE in both patients and controls (Table 1). For the *p21* C98A polymorphism, the AA was associated with a significantly increased risk of meningioma compared with CC genotype ($p = 0.02$) (Table 2). In the dominant model, there was a significantly increased risk of meningioma in subjects carrying A allele (AA+CA), when compared with homozygote CC. The frequency of A allele was significantly higher in the case group than in the controls ($p = 0.00008$), suggesting that A allele is risk factor that could increase meningioma susceptibility. For *p21* (C70T), subjects carrying CT were approximately twice as likely to develop meningioma compared to the CC genotype. Subsequent grouping of the CC and TT genotypes in the over-dominant model revealed a significantly increased risk of meningioma in CT genotype carriers when compared with others. In the analysis of allele distributions, the T allele conferred an increased risk of meningioma ($p = 0.01$).

The main roles of *p21* include repairing DNA damage, regulating the cell cycle, with tumour growth suppression, and a block on DNA replication [11,12]. Two functional polymorphisms of *p21*, C98T and C70T, were associated with an altered activity of *p21* [4,5]. These might be the molecular mechanisms of the important roles of *p21* C98A and C70T polymorphisms in meningioma. The expression of the A allele of C98A polymorphism does not affect the previously reported tumour suppressor activity of *p21* [13]. Several population-based studies have reported a prevalence ranging from 6 to 57% for the C98A mutant allele (A allele) and from 5 to 48% for the C70T mutant allele (T allele) among different populations. The *p21* C98A and C70T polymorphisms have been identified by their association with the risk of cancer, but the results from these studies are controversial. Shao et al. have reported that *p21* rs1059234 TT, *p21* rs3176352 GC/CC, *p21* rs762623 GA and *TP53BP1* rs560191 CC genotypes are associated with the development of gastric adenocarcinoma [14]. However, a case-control study by Gomes et al. showed no significant association between the *p21* C98A polymorphism and oral squamous cell carcinoma risk [15]. These inconsistent results may be due to variation in phenotype definition and control selection, limited power (due to their small sample size) and possible confounding by population substructure.

The limitations encountered in this study were as follows. First, the number of samples is relatively small. However, this study has enough statistical power to generate valid results. Second, only two SNPs of *p21* were investigated in the present study, which did not cover all SNPs. Third, environmental risk factors were not available for further analysis, which should be investigated in future studies.

This work represents an advance in biomedical science because it indicates that *p21* C98A and C70T

Table 1. Hardy–Weinberg analysis.

Polymorphic site	Population	Genotype	*n*(%)	Genotype frequency	Expected frequency	HWE *p*-value
p21 C98A	Cases	CC	154(68.4)	0.68	0.67	0.11
		CA	60(26.7)	0.27	0.30	
		AA	11(4.9)	0.05	0.03	
	Controls	CC	262(81.9)	0.82	0.81	0.08
		CA	52(16.2)	0.16	0.18	
		AA	6(1.9)	0.02	0.0	
p21 C70T	Cases	CC	138(61.3)	0.61	0.63	0.14
		CT	81(36)	0.36	0.33	
		TT	6(2.7)	0.03	0.04	
	Controls	CC	234(73.1)	0.73	0.72	0.21
		CT	76(23.7)	0.24	0.25	
		TT	10(3.1)	0.03	0.02	

Table 2. Genotype frequencies of p21 C98A and C70T polymorphisms in cases and controls.

Genetic models		Genotypes	Cases *n*(%)	Controls *n*(%)	OR(95%CI)	*p*-value
p21 C98A Codominant		CC	154(68.4)	262(81.9)	1.00	
		CA	60(26.7)	52(16.2)	1.96(1.28–2.99)	0.001
		AA	11(4.9)	6(1.9)	3.11(1.13–8.60)	0.02
Dominant		CC	154(68.4)	262(81.9)	1.00	
		AA+CA	71(31.5)	58(18.1)	2.08(0.39–3.10)	0.0003
Recessive		CA+CC	214(97.3)	314(98.1)	1.00	
		AA	11(4.9)	6(1.9)	2.69(0.98–7.38)	0.05
Overdominant		CC+AA	165(73.3)	268(83.7)	1.00	
		CA	60(26.7)	52(16.2)	2.69(0.98–7.38)	0.003
p21 C70T Codominant		CC	138(61.3)	234(73.1)	1.00	
		CT	81(36)	76(23.7)	1.80(1.23–2.63)	0.002
		TT	6(2.7)	10(3.1)	1.01(0.36–2.86)	0.97
Dominant		CC	138(61.3)	234(73.1)	1.00	
		TT+CT	87(38.7)	86(27)	1.71(1.19–2.47)	0.003
Recessive		CT+CC	219(97.3)	310(96.8)	1.00	
		TT	6(2.7)	10(3.1)	0.84(0.30–2.37)	0.75
Overdominant		CC+TT	144(64)	244(76.2)	1.00	
		CT	81(36)	76(23.7)	1.80(1.24–2.62)	0.002
Allele						
C98A	C70T					
C	C		342(76)	523(81.7)	1.00	
A	C		15(3.3)	21(3.3)	1.09(0.55–2.14)	0.79
C	T		26(5.8)	53(8.3)	0.75(0.46–1.22)	0.24
A	T		67(14.9)	43(6.7)	2.38(1.58–3.57)	**<0.001**

Notes: OR, Odds Ratio; 95%CI, 95% Confidence Interval; Boldfaced value indicates a significant difference at the 5% level.

polymorphisms may play an important role in the susceptibility to meningioma and so aid diagnosis.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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