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Allelic and Haplotype Frequencies of the p53 Polymorphisms in Brain Tumor Patients

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Summary

The polymorphisms of the tumor suppressor gene p53 in exon 4 (p53 BstUI) and in intron 6 (p53 MspI) have been suggested to be associated with the genetically determined susceptibility in diverse types of human cancer. In our hospital-based case-control study, we examined the allele and genotype incidence of these polymorphisms as well as their haplotype combinations in 60 brain tumor patients (27 males and 33 females) and 183 controls without malignancies. The genotype characteristics were determined by the PCR-based RFLP method using DNA extracted from peripheral blood. In this study we show that the p53 BstUI and the p53 MspI polymorphisms are not associated with increased risk of brain tumors. Thus, we conclude that the p53 BstUI and the p53 MspI polymorphic sites within the tumor suppressor gene p53 do not represent genetic determinants of susceptibility to brain tumors.

Key words

Brain tumor • Susceptibility • p53 • Polymorphism

Introduction

The concept of neoplastic transformation links together two types of genetic background of cancer with the same final results. Besides entirely somatic cell-gene deregulation, a genetically determined susceptibility is taken into account. The predisposition to cancer results from inheritance of altered alleles of genes, which are usually of the tumor suppressor type. The highly significant tumor suppressor gene, p53, is implicated in a wide range of human cancers, including brain tumors. A mutated p53 gene is found in approximately 25 % of human gliomas (Greenblatt *et al.* 1994). Somatic mutations occur mainly in astrocytomas (Dalrymple *et al.* 1994, Goussia *et al.* 2000) but they are less common in non-astrocytic brain tumors (Von Deimling *et al.* 1992). On the other hand, germ line mutations within the p53 gene are associated with the Li-Fraumeni syndrome (LFS), an autosomal dominant trait associated with a predisposition to develop cancers in several tissues at a high frequency (Malkin 1993). LFS patients are particularly prone to carcinomas of the breast and adrenal cortex, sarcomas of the soft tissues and bone, acute leukemia, and brain tumors.

The wild-type p53 gene exhibits several polymorphisms both in coding and non-coding regions, namely single nucleotide polymorphisms (SNPs) in exon 4 at codon 72 (Harris *et al.* 1986, Matlashewski *et al.*

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1987), and in intron 6 (Peller *et al.* 1995). The p53 codon 72 SNP (p53 BstUI) causes amino acid replacement of proline (CCC, A1 allele) by arginine (CGC, A2 allele). Thomas *et al.* (1999) reported that, although both p53 BstUI variants are wild type, they are functionally distinct because of inequalities in both their biological and biochemical properties. The p53 intron 6 (p53 MspI) SNP represents a polymorphic site within the non-coding region of the p53 gene carrying two alleles, allele A1 and allele A2. The present study is based on evaluation of these two p53 SNPs as putative molecular markers of inherited predisposition to brain tumors.

Methods

Blood samples were collected from 60 patients (27 males and 33 females) with histologically proven diagnosis of brain tumor recruited at the Department of Neurosurgery, Teaching Hospital, Košice. The average age of patients was 51.2 years (range 22-77 years of age). Sixty brain cancer patients were histologically classified as 34 meningiomas and 26 astrocytomas. Out of the 60 brain cancer patients, 23 (about 38 %) were smokers. Blood samples of 183 healthy donors (111 males and 72 females) were collected as controls. The control group was age adjusted according to patients, range 22-79 years of age. Out of the 183 population controls, 56 (about 31 %) were smokers. All cases and controls were of Slovak origin (Caucasians) from different regions of Eastern Slovakia. Genomic DNA was isolated from 1-5 ml peripheral blood samples by salting-out method (Miller *et al.* 1988).

Genotyping of the p53 exon 4 codon 72 SNP (p53 BstUI) and the p53 intron 6 SNP (p53 MspI) were performed as described previously (Själander *et al.* 1995, Wang-Gohrke *et al.* 1998). Briefly, the p53 exon 4 fragment encompassing the codon 72 and the p53 intron 6 fragment were amplified by touchdown PCR resulting in a 318 bp and 240 bp PCR products. An aliquot of PCR products (about 10 %) were digested with BstUI and MspI restriction enzymes (New England BioLabs, UK) at 60 ^{o}C for 2 hours and 37 ^{o}C for 16 hours, electrophoresed through 10 % polyacrylamide gel for 2.5 hours at 150 V and the DNA fragments were visualized with ethidium bromide (Fig. 1).

The odds ratio (OR) and its 95 % confidence interval (95 % CI) were used to analyze the frequencies of alleles and genotypes; the corresponding P values were calculated. A difference was determined significant for

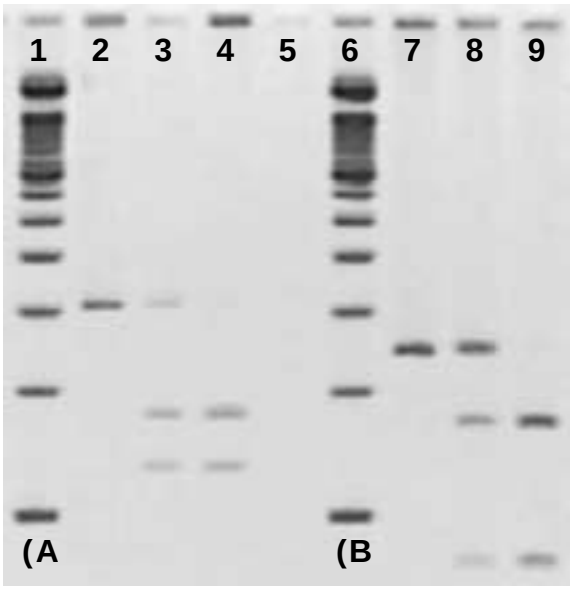

Fig. 1. *Polyacrylamide gel electrophoresis of the p53 BstUI and the p53 MspI PCR products digested with BstUI and MspI restriction enzymes. (A): A1 allele does not create BstUI restriction site. The PCR product has 318 bp. Presence of CGCC sequence in A2 allele creates BstUI restriction site which leads to two fragments 182 bp and 136 bp. (B): A1 allele does not create MspI restriction site. The PCR product has 240 bp. Presence of CCGG sequence in A2 allele creates MspI restriction site which leads to two fragments 164 bp and 76 bp. Lanes 1, 6: 100 bp DNA ladder; lanes 2, 7: 1-1 homozygotes; lanes 3, 8: 1-2 heterozygotes; lanes 4, 9: 2-2 homozygotes; lane 5: no DNA in PCR reaction (blank).*

$P<0.05$. Logistic regression models for case-control analysis were used to adjust for smoking and gender and to estimate OR and its 95 % CI. Pearson's χ^2 test or Fisher's exact test were used for evaluating differences of alleles, genotypes, and haplotypes frequencies between cases and controls, and to identify deviations from the Hardy-Weinberg proportion (χ^2HW). All computations were undertaken using statistical software ARCUS QUICKSTAT Biomedical Version 1.1 (Addison Wesley Longman Ltd, UK). Haplotype frequencies were estimated according to the principles outlined by Hill (1974).

Table 1. p53 genotypes and A1 and A2 allele frequencies in brain cancer patients and controls

Polymorphism		p53 genotype			Allele frequency		χ^2_{HW}	n
		A1/A1	A1/A2	A2/A2	A1	A2		
p53 BstUI	*Controls*	25	72	86	0.33	0.67	2.45	183
	Patients	6	[c]25	29	[a]0.31	0.69	0.03	60
p53 MspI	*Controls*	3	47	133	0.14	0.86	0.33	183
	Patients	1	[d]16	43	[b]0.15	0.85	0.13	60

Differences of allele frequencies: Chi-square test. Patients vs. Controls: [a]P=0.61 (OR 0.67; 95 % CI 0.51-0.87), [b]P=0.89 (OR 0.73; 95 % CI 0.50-1.07). Differences of genotypes distributions: Chi-square test. Heterozygous vs. Homozygous: [c]P=0.75 (OR 0.75; 95 % CI 0.53-1.05), [d]P=0.88 (OR 0.75; 95 % CI 0.50-1.14)

Table 2. Double genotype combinations of the p53 polymorphisms in brain cancer patients and controls

p53 BstUI	p53 MspI	Controls	Patients
A2/A2	*A2/A2*	85 (46.5 %)	28 (46.6 %)
A1/A2	*A2/A2*	37 (20.2 %)	13 (21.7 %)
A1/A2	*A1/A2*	35 (19.1 %)	12 (20.0 %)
A1/A1	*A2/A2*	11 (6.0 %)	2 (3.3 %)
A1/A1	*A1/A2*	11 (6.0 %)	3 (5.0 %)
A1/A1	*A1/A1*	3 (1.6 %)	1 (1.7 %)
A2/A2	*A1/A2*	1 (0.6 %)	1 (1.7 %)
Total		183 (100.0 %)	60 (100.0 %)

Patients vs. Controls: P=0.40 (Fisher's exact test)

Results

The incidence of the p53 BstUI and p53 MspI genotypes and alleles polymorphisms in brain tumor patients and in the controls are presented in Table 1. Both patients and the controls showed a generally good fit to Hardy-Weinberg equilibrium. In the p53 BstUI polymorphism, no significant A1 allele frequency differences were found between patients and the controls (P=0.61, OR 0.67, 95 % CI 0.51-0.87). The p53 MspI A1 allele frequency was almost identical in brain tumor patients and population controls (P=0.89, OR 0.73, 95 % CI 0.50-1.07). No significant differences were found with reference to the genotype distribution.

The genotype characteristics of two p53 polymorphisms were analyzed together in combination for each individual. No significant differences were found between patients and the controls by Fisher's exact test, P=0.40 (Table 2). Of the seven determined combinations, three were common to both patients and the controls (p53 BstUI-p53 MspI: A2/A2-A2/A2, A1/A2-A2/A2, A1/A2-A1/A2). The remaining four combinations were less frequent with a total percentage of about 12 % in brain tumor patients, and about 14 % in healthy controls. There were found differences between patients and controls with respect to the frequency of the genotype combinations A1/A1-A2/A2 (3.3 % vs. 6.0 %) and A2/A2-A1/A2 (1.7 % vs. 0.6 %). However, these

categories were too small to allow meaningful statistical analysis.

Table 3 presents an estimation of the haplotype frequencies in brain tumor patients and the control group. No significant (P=0.35) differences were found in patients from the control group. The haplotype combination A2-A1 appeared to be approximately three times more frequent in brain tumor patients than in the controls (0.0129 *vs.* 0.0046), although no statistical significance was observed due to the small number of subjects.

Table 3. Estimated haplotype frequencies between the p53 BstUI and the p53 MspI polymorphisms in brain cancer patients and controls

p53 BstUI-p53 MspI	Estimated haplotype frequency				Number of alleles
	A1-A1	A1-A2	A2-A1	A2-A2	
Controls	0.1402	0.1931	0.0046	0.6621	366
Patients	0.1371	0.1712	0.0129	0.6788	120

Patients vs. Controls: P=0.35 (Fisher's exact test)

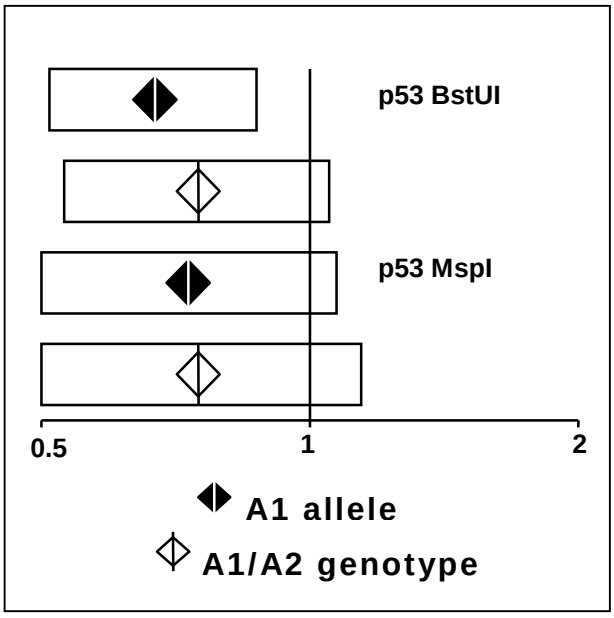

Fig. 2. *Estimated ORs and their 95 % CIs for the differences in A1 allele and A1A2 genotype frequencies between patients and controls (adjusted for gender and smoking). No significant differences were found both in allele and genotype frequencies with respect to the p53 BstUI (P=0.61, P=0.75) and the p53 MspI (P=0.89, P=0.88) polymorphisms*

Discussion

The genes involved in tumorigenesis are potential molecular markers associated with cancer susceptibility. Therefore, we investigated the relationship between the p53 BstUI and the p53 MspI polymorphisms within the tumor suppressor gene p53 and the susceptibility to brain tumors.

In our previous case-control study, a decreased frequency of the p53 MspI A1/A2 genotype was found in bladder cancer (Biroš *et al.* 2000). More recently, both Wang *et al.* (1999) and Kawajiri *et al.* (1993) in an earlier study reported a relationship between the p53 BstUI A1/A1 genotype and lung cancer in Asiatic populations of Taiwan and Japan. In studies from Sweden of nasopharyngeal cancer and Germany of breast cancer, significant associations of the p53 BstUI A1/A1 and the p53 MspI A1/A2 genotypes were observed (Birgander *et al.* 1996, Wang-Gohrke *et al.* 1998). The consistent association between the p53 SNPs (p53 BstUI and p53 MspI) and various forms of human malignancies may serve as arguments in favor of these polymorphic sites as genetic determinants of susceptibility. However, the current results of brain tumor patients differed from those previously found in bladder, lung, nasopharyngeal, and breast cancers.

In our present study, the genotype distributions of the p53 BstUI and the p53 MspI polymorphisms were found to be in Hardy-Weinberg equilibrium both in brain tumor patients and healthy controls. The genotype analysis of patients and the controls did not reveal any significant differences in allelic distribution of the p53 BstUI and the p53 MspI polymorphisms. This observation was confirmed by the genotype estimation (Fig. 2). Admittedly, searching for genotype combinations of these two polymorphisms displayed very

similar incidence of the p53 BstUI-p53 MspI combined genotypes in patients and the controls. As can be seen in Table 2, the most frequent combination was A2/A2-A2/A2, which occurred in about 47 % of cases in both patients and the controls). Consequently, the most common ('wild-type') haplotype combination is A2-A2, i.e. the p53 BstUI A2 allele (or Arg allele) linked to presence of the MspI restriction site. The next haplotype in frequency (A1-A2) differs from the 'wild type' in only one mutation, and the third haplotype in frequency (A1-A1) contains two mutational events with respect to the 'wild-type'. However, the haplotype frequencies in brain tumor patients were similar to controls. It can be hypothesized that the lack of association between these two p53 SNPs and brain tumors is likely due to the different molecular etiology of brain malignancies in comparison to some of previously studied. Although tumor mutations are heterogeneous, the majority of p53 somatic point mutations in brain tumors are reported to be transitions, including a high portion of G:C→A:T, especially at CpG dinucleotides in codons of conserved residues (~38 %). On the other hand, G:C→A:T transitions of other tumor sites are found in only ~11 % of lung, ~18 % of bladder, and ~21 % of breast tumors (Hollstein *et al.* 1996). Thus, the heterogeneity of mutational patterns among human tumors may be the reason for the absence of linkage disequilibria between the p53 BstUI and/or the p53 MspI types and some susceptible site within the p53 gene, as it was shown in our study of brain tumors.

Summarizing our data concerning the incidence of alleles, genotypes, and haplotypes of the p53 BstUI and the p53 MspI SNPs in patients and the controls, we can conclude that these polymorphic sites within the tumor suppressor gene p53 do not represent the major genetic determinants of susceptibility to brain tumors.

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