Association of *p53* and *p21* polymorphisms with prostate cancer

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Received May 19, 2015; Accepted July 3, 2015

DOI: 10.3892/br.2015.496

Abstract. Cell cycle deregulation is common in human cancer. Alterations of the tumor-suppressor gene *p53* and its downstream effector p21 have been indicated in the development of numerous human malignancies. Therefore, we hypothesize that the *p53* codon 72 polymorphism, either on its own or in combination with *p21* (*C98A* and *C70T*) polymorphisms, modifies the risk of prostate cancer within the Slovak population, and no previous studies have investigated these gene-gene interactions in the pathogenesis of prostate cancer in the Slovak population. Polymerase chain reaction-restriction fragment length polymorphism was used to determine the *p53* and *p21* genotypes in subjects comprising 300 prostate cancer patients and 446 healthy individuals. These 3 polymorphisms individually did not correlate with the prostate cancer risk. Conversely, the interaction between the *p53* and *p21* polymorphisms significantly decreased the risk of prostate cancer, with the odds ratio (OR) being 0.49 [95% confidence interval (CI), 0.27-0.86; P<0.05] for subjects carrying the *p53* codon 72 arginine (*Arg*)/proline *(Pro)+Pro*/*Pro* and *p21 C98A* CA genotypes compared to the combined reference genotypes p53 codon 72 *Arg*/*Arg* and *p21 C98A* CC. Neither the *p53* genotypes nor the *p21* genotypes showed statistically significant differences in Gleason score or serum prostate-specific antigen levels (P>0.05). A decreased risk of prostate cancer association with the *p21 C98A CA* genotype (OR=0.58; 95% CI, 0.36-0.93; P<0.05) in non-smokers compared to the non-smokers with the *p21 C98A* CC genotype was observed. Smokers carrying the *p53* codon 72 *Pro*/*Pro* genotype were not at any significant risk of prostate cancer (OR=2.97; 95% CI, 0.51-17.15) compared to the non-smokers with the *Arg*/*Arg* genotype. Taken together, to the best of our knowledge this is the first study to show that a combination of the variant genotypes of *p53* codon 72 and *p21 C98A* may modify the prostate cancer risk within the Slovak population.

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Key words: *p53*, *p21*, prostate cancer, cigarette smoking, Slovak population

Introduction

Prostate cancer is the most frequently diagnosed cancer in males in numerous countries, including Slovakia (1). It is a multifactorial and polygenic disease in origin and reflects hereditary and environmental components (2-5). Accumulating evidence indicates that genetic variants of an increasing number of genes, including tumor-suppressor genes, may modulate the risk for prostate cancer (6). One of these is the gene for the tumor-suppressor protein p53. The *p53* gene comprises 11 exons and is located on the short arm of chromosome 17p13. It encodes a 1.53 kDa nuclear phosphoprotein, composed of 393 amino acids, which is highly conserved in diverse organisms (7,8). The p53 protein is a tetrameric transcription factor that regulates the expression of a wide variety of genes involved in cell cycle arrest and apoptosis in response to genotoxic or cellular stress.

A key target gene executing the role of p53 in cell cycle arrest is *p21*, which is induced by p53 through direct binding to the *p21* promoter (9-11). The *p21* gene is localized on chromosome 6p21.2, comprising 3 exons and 2 introns, and encodes the p21 protein. The p21 protein is also known as cyclin-dependent kinase (CDK) inhibitor 1A, WAF1, CAP20, Cip1 and Sdi1 (9). It binds tightly to complexes of cyclins and CDKs (CDK2, CDK3, CDK4 and CDK6), inhibiting their function. Accordingly, induction of p21 arrests the cell cycle in the G_1 phase, and in the process, mediates the function of p53 in preventing the division of DNA-damaged cells (12).

p53 is the most frequently mutated gene in human tumors, with >50% of tumors harboring mutations in this gene (7,13). At least 13 single-nucleotide polymorphisms (SNPs) of the *p53* gene have been described. The most commonly studied among these is codon 72 polymorphism in exon 4, which leads to a

G to C transversion [substitution of arginine (Arg) by proline (Pro); rs1042522]. This type of *p53* polymorphism, found in the general population, produces a marked change in the structure of the p53 protein (14). The 2 polymorphic forms of *p53* may result in an evident change of the primary structure of the protein, modifying its biochemical properties and effects. The *Pro72* variant interacts more effectively with elements of the transcriptional machinery and induces higher levels of transcriptional activity compared with the *Arg72* form. The *Pro72* variant also induces G_1 arrest and more effectively activates DNA repair system (15-18). However, *Arg72* has shown apoptotic induction with faster kinetics and suppresses transformation more efficiently compared with the *Pro72* variant (15). Thus, the differences in these biological activities caused by each of the 2 polymorphic variants may modify the risk of cancer (19). *Pro* is the ancestral allele in comparison to the *Arg* allele, as shown by a 95% allele frequency in the primitive African population, and the fact that the frequency of the I allele progressively increased as populations migrated further North (80% allele frequency in Northern Europe) (20).

The association of the *p53* codon 72 polymorphism with prostate cancer risk has been investigated by several studies. The two variants were considered wild-type, resulting in a non-conservative change (6,15). In certain studies the *Pro* allele was associated with increased prostate cancer risk (21-25), while in others the *Arg* allele was associated with prostate cancer predisposition (26). Other studies, mainly larger studies and meta-analyses, did not detect any association of the *p53* codon 72 polymorphism with prostate cancer risk (27-30).

Polymorphisms of the *p21* gene results in altered transcripts and suppressed apoptosis. In the *p21* gene a total of 40 SNPs have been identified, in addition to 2 major *p21* polymorphisms in codon 31 *(p21 C98A*, rs1801270) and in the 3' untranslated regions (3' UTRs) (*p21 C70T*, rs1059234). Thus, independently or in combination, they may have an effect on carcinogenesis (31,32). In the *p21 C98A* polymorphism, a substitution of C to A in the third base of codon 31 results in a serine (Ser) to Arg substitution in the DNA-binding zinc finger motif of the protein (33). *p21 C70T* causes a single C to T substitution 20 nucleotides (nt) downstream of the stop codon at exon 3. Therefore, an alteration in the p21 functional and/or promoter regions may adversely affect the regulation of cellular proliferation and increase susceptibility to cancer (34).

As p53 and p21 physically and functionally interact in the p53 pathway, the *p21 C98A* and *p21 C70T* polymorphisms were selected together with the well-studied p53 codon 72 polymorphism to test our hypothesis that these SNPs, through synergistic effects, are associated with the risk of prostate cancer. Furthermore, to the best of our knowledge, no previous studies have investigated the possible role of these three gene polymorphisms and their gene-gene interactions in the pathogenesis of prostate cancer in the Slovak population.

Materials and methods

Study subjects and samples. A total of 746 unrelated subjects (Caucasians), living in the north of Slovakia, were enrolled in the study from the Department of Urology, Comenius University in Bratislava, Jessenius Faculty of Medicine and UHM (Martin, Slovakia), between 2005 and 2011. All the prostate cancer patients (n=300) were diagnosed histopathologically, and by reviewing the medical records it was confirmed that they had no prior history of other cancers. Healthy controls (n=446) consisted of randomly-selected volunteers. Cases and controls were tested for serum prostate-specific antigen (PSA) levels (PSA chemiluminescence immunoassay), and men with abnormal PSA levels were omitted from the normal controls or received further examination, including prostate biopsy, to rule out any prostatic disease conditions. Prior to enrolment, peripheral blood was obtained from every individual. A detailed description of the inclusion and exclusion criteria in the case and control groups was as previously reported (4). A standard questionnaire obtained by face-to-face interviews using trained interviewers formed the basis of collection of demographic data and the associated factors, including age, smoking history (i.e. habitual smokers, those who have never smoked, or never smokers) and family history of cancer. The study was approved by the Ethics Board of Jessenius Faculty of Medicine, Comenius University and informed written consent was obtained from all the individuals prior to initiation. The studied population is described in Table I.

Table I. Characteristics of the prostate cancer patients and healthy controls.

Characteristics	Cases (n=300)	Controls (n=446)	P-value
Age, years			
Mean ± SD	65.8±7.7	62.2±8.5	<0.001
Median (IQR)	65 (60-72)	61 (55-68)	
PSA, ng/ml			
Mean ± SD	10.0±9.1	1.23±1.00	<0.001
Median (IQR)	7.38 (4.68-13)	0.86 (0.47-1.74)	
Gleason score			
Mean ± SD	6.88±1.25	NA	
Median (IQR)	7 (6-7)	NA	
Smoking status, no. (%)			
Never smokers	202 (67.33)	325 (72.87)	NS
Smokers	76 (25.33)	105 (23.54)	
Unknown	22 (7.33)	16 (3.59)	

NA, not applicable; NS, not significant; SD, standard deviation; IQR, interquartile range.

DNA extraction and polymorphism genotyping. Genomic DNA was extracted from peripheral blood lymphocytes using the conventional phenol-chloroform method. The polymorphic sites of the *p53* codon 72, *p21 C98A* and *p21 C70T* genes were genotyped by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay (35,36). Previously reported primers and restriction enzymes in PCR-RFLP are listed in Table II.

The 199-base pair (bp) PCR product of the *p53* codon 72 polymorphism was digested with 2 units of the fast digest restriction enzyme *Bsh*1236I (Fermentas Co., St. Leon-Rot, Germany) at 37˚C for 30 min and separated on ethidium-bromide-stained

Table II. Primer sequences and restriction enzymes used for the study of the *p53* and *p21* gene polymorphisms.

Genes polymorphisms	Primers	Restriction enzyme
p53		
Arg72Pro	F: 5'-TTGCCGTCCCAAGCAATGGATGA-3'	FastDigest
	R: 5'-TCTGGGAAGGGACAGAAGATGAC-3'	*Bsh*1236I
p21		
C98A	F: 5'-GTCAGAACCGGCTGGGGATG-3'	FastDigest
	R: 5'-CTCCTCCCAACTCATCCCGG-3'	*Bpu*1102I
C70T	F: 5'-CCCAGGGAAGGGTGTCCTG-3'	FastDigest
	R: 5'-GGGCGGCCAGGGTATGTAC-3'	*Pst*I

Arg, arginine; Pro, proline; F, forward; R, reverse.

3% agarose gel. When the *Bsh*1236I restriction site (*Arg* allele) was present, the 199-bp fragment was digested into two 113- and 86-bp fragments. The *Pro* allele was not cleaved by *Bsh*1236I, and had a single band of 199 bp. The heterozygous genotype (*Arg*/*Pro*) had 3 bands (199, 113 and 86 bp).

The 272-bp PCR product of *p21 C98A* was subsequently digested with 2 units of the fast digest *Bpu*1102I restriction enzyme (Fermentas Co.) at 37˚C for 20 min and separated on ethidium-bromide-stained 2% agarose gel. Digestion of the wild-type allele (CC) created DNA fragments of 89 and 183 bp, whereas the AA allele, which lacks a *Bpu*1102I site, yielded the original 272-bp fragment.

The 298-bp PCR product of *p21 C70T* was digested with 2 units of the fast digest restriction enzyme *PstI* (Fermentas Co.) at 37˚C for 30 min and separated on ethidium-bromide-stained 3% agarose gel. The intact *Pst*I site (in the wild-type of the allele) generated two 126- and 173-bp fragments. The loss of the *Pst*I site (C to T polymorphism) yielded a 298-bp fragment.

All genotypes were verified by repeating PCR-RFLP on 50 random samples.

Statistical analysis. Mann-Whitney U test was used to analyse the differences between the controls and cases in age and PSA level. Homogeneity of the sample with respect to the observed genotype counts and those expected under Hardy-Weinberg equilibrium was tested using the exact method (37). Associations between two categorical variables were assessed using Fisher's exact test and odds ratios (ORs) with ~95% confidence intervals (CIs). All the presented P-values are two-sided. P<0.05 was considered to indicate a statistically significant difference. Statistical analysis was performed using statistical software StatsDirect 2.8.0 (http://www.statsdirect.com).

Results

Subject characteristics. The demographic characteristics and the clinical information of 300 prostate cancer patients and 446 controls in the study are outlined in Table I. Briefly, there was a significant difference in terms of distribution of age and serum PSA levels between the cases and controls (P<0.001). However, in comparison with the controls, a higher proportion of the prostate cancer patients smoked (25.33 vs. 23.54%, P>0.05). The percentage of Gleason score ≤7 and >7 was 56 and 17%, respectively. For 27% of these 300 patients, the final pathological grade was not included in the analysis as the grading had been performed using different grading systems.

Genotype distribution. The genotype distribution of the studied SNPs in the cases and controls and their associations with prostate cancer risk are summarized in Table III. The distributions of the genotypes of these genetic polymorphisms in the controls were in Hardy-Weinberg equilibrium (P>0.05), except the genotypes of *p53* codon 72 and *p21 C98A*. Cases had the highest frequency of wild-type *p53* codon 72 (*Arg*/*Arg*), wild-type *p21 C98A* (CC) and heterozygous genotype *p21 C70T* (CT). Controls had the highest frequency of heterozygous genotype of *p53* codon 72 (*Arg*/*Pro*), heterozygous genotype of *p21 C98A* (CA) and wild-type *p21 C70T* (CC) genotype. In the two groups, no mutant genotype of *p21 C98A* (AA) and *p21 C70T* (TT) was identified. No significant difference in the genotype frequencies of the studied SNPs was found between the cases and controls (P>0.05). None of the variant genotypes alone was associated with a significantly altered risk. The *p53* codon 72 *Pro*/*Pro* genotype was not associated with altered prostate cancer risk (OR=1.08; 95% CI, 0.47-2.44; P>0.05) when compared with the *Arg*/*Arg* genotype. The *p21 C98A* CA and *p21 C70T* CT genotypes appeared to be associated with non-significantly reduced/no-change prostate cancer risk (OR=0.73; 95% CI, 0.50-1.06 and OR=1.06; 95% CI, 0.72-1.58, respectively).

Combined effect of the polymorphisms. The combined effect of *p53* codon 72, *p21 C98A* and *p21 C70T* polymorphisms on the risk of prostate cancer was further evaluated. As shown in Table IV, the combined risk *p53* codon 72 *Arg*/*Pro+Pro*/*Pro* and *p21 C98A CA* genotypes was found to be associated with a significant 51% reduction of prostate cancer risk (OR=0.49; 95% CI, 0.27-0.86; P<0.05) relative to *p53* codon 72 *Arg*/*Arg* and *p21 C98A* CC genotypes. The combination of the *p53* codon 72 *Arg*/*Pro+Pro*/*Pro* genotype and *p21 C70T* heterozygous mutant genotype (CT) showed a non-significant decrease of prostate cancer risk (OR=0.70; 95% CI, 0.40-1.22; P>0.05) compared to the combined reference genotypes *p53* codon 72 (*Arg*/*Arg*) and *p21 C70T* (CC). The individuals with combination genotypes of *p53* codon 72 *Arg*/*Arg* and *p21 C70T* CT were shown to have a 1.74-fold increased prostate cancer risk

Table III. Genotype frequencies of *p53* codon 72, *p21C98A* and *p21 C70T* among cases and controls and their association with the risk of prostate cancer.

Genotype	Cases, n (%)	Controls, n (%)	OR (95% CI)	P-value
p53 Codon 72				
Arg/Arg	146 (48.67)	200 (44.84)	1.00 (ref.)	
Arg/Pro	143 (47.67)	232 (52.02)	0.84 (0.63-1.14)	NS
Pro/Pro	11 (3.67)	14 (3.14)	1.08 (0.47-2.44)	NS
Arg/Pro+Pro/Pro	154 (51.33)	246 (55.16)	0.86 (0.64-1.15)	NS
p21 C98A				
CC	250 (83.33)	350 (78.48)	1.00 (ref.)	
CA	50 (16.67)	96 (21.52)	0.73 (0.50-1.06)	NS
p21 C70T				
CC	249 (83.00)	374 (83.86)	1.00 (ref.)	
CT	51 (17.00)	72 (16.14)	1.06 (0.72-1.58)	NS

NS, not significant; Arg, arginine; Pro, proline; OR, odds ratio; CI, confidence interval.

Table IV. ORs and 95% CIs for the interaction between *p53* codon 72 and *p21 C98A* or *p21 C70T* polymorphisms in prostate cancer.

	p21 C98A				*p21 C70T*			
	CC		CA		CC		CT	
Polymorphism	Cases/ controls, n	OR (95% CI)	Cases/ controls, n	OR (95% CI)	Cases/ controls, n	OR (95% CI)	Cases/ controls, n	OR (95% CI)
p53 codon 72								
Arg/Arg	116/163	1.00 (ref.)	30/37	1.14 (0.67-1.95)	117/175	1.00 (ref.)	29/25	1.74 (0.97-3.11)
Arg/Pro +Pro/Pro	134/187	1.01 (0.73-1.39)	19/55	0.49 (0.27-0.86)[a]	132/199	0.99 (0.72-1.37)	22/47	0.70 (0.40-1.22)

[a]P<0.05. OR, odds ratio; CI, confidence interval; Arg, arginine; Pro, proline.

(95% CI, 0.97-3.11; P>0.05) in comparison to the combined reference genotypes *p53* codon 72 (*Arg/Arg*) and *p21 C70T* (CC).

Association of the polymorphisms and clinicopathological characteristics. To further evaluate the influence of the *p53* and *p21* genotypes on the severity of prostate cancer, the association between these polymorphism and the clinicopathological characteristics of prostate cancer patients (Gleason score and serum PSA levels) was investigated. There was no evidence for an association of the *p53* and *p21* genotypes with aggressiveness of the tumor when comparing cases with Gleason grade ≤7 with those >7 (P>0.05; data not shown). Stage data were not available for analysis. Similar results were obtained when comparing cases with serum PSA levels <10 ng/ml with those ≥10 ng/ml (P>0.05; data not shown).

The association between these 3 polymorphisms and smoking status was also examined (Table V). Smokers carrying the *p53* codon 72 *Pro/Pro* genotype were at no significantly increased risk of prostate cancer (OR=2.97; 95% CI, 0.51-17.15; P>0.05) compared to non-smokers with the *Arg/Arg* genotype. Among non-smokers with *Arg/Pro* and *Pro/Pro* genotypes, the risk of prostate cancer was decreased (OR=0.81; 95% CI, 0.56-1.15 and OR=0.92; 95% CI, 0.34-2.44; P>0.05; respectively). By contrast, a significant association was found in non-smokers carrying the *p21 C98A* CA genotype (OR=0.58; 95% CI, 0.36-0.93; P<0.05) compared to non-smokers with the CC genotype. For the *p21 C70T* CT genotype, there was no significant change in the risk of prostate cancer among non-smokers and smokers (OR=1.13; 95% CI, 0.70-1.80; and OR=0.69; 95% CI, 0.30-1.57; P>0.05; respectively) in comparison to the CC genotype in non-smokers.

Discussion

The present study investigated the association of prostate cancer risk with the *p53* codon 72, *p21 C98A* and *p21 C70T* polymorphisms in the Slovak population. To the best of our knowledge, no previous studies addressed the correlation of these 3 polymorphisms with prostate cancer risk. Previous studies only investigated one or two of these polymorphisms

Table V. Association between the *p53* and *p21* polymorphisms and smoking status.

	Non-smokers		Smokers	
Polymorphism	Cases/controls, n	OR (95% CI)	Cases/controls, n	OR (95% CI)
p53 codon 72				
Arg/Arg	98/141	1.00 (ref.)	33/49	0.97 (0.58-1.62)
Arg/Pro	97/173	0.81 (0.56-1.15)	39/54	1.07 (0.59-1.96)
Pro/Pro	7/11	0.92 (0.34-2.44)	4/2	2.97 (0.51-17.15)
Arg/Pro+Pro/Pro	104/184	0.81 (0.57-1.16)	43/56	1.14 (0.63-2.06)
p21 C98A				
CC	172/250	1.00 (ref.)	63/86	1.06 (0.73-1.55)
CA	30/75	0.58 (0.36-0.93)[a]	13/19	0.87 (0.40-1.88)
p21 C70T				
CC	167/274	1.00 (ref.)	66/86	1.56 (0.87-1.83)
CT	35/51	1.13 (0.70-1.80)	10/19	0.69 (0.30-1.57)

[a]P<0.05. OR, odds ratio; CI, confidence interval; Arg, arginine; Pro, proline.

in prostate cancer patients. Although each polymorphism individually was not associated to prostate cancer risk, the combination of the *p53* codon 72 *Arg/Pro+Pro/Pro* and *p21 C98A* CA genotypes was associated with a significant 51% decreased risk of prostate cancer. Furthermore, in the present study, non-smoking individuals were significantly decreased risk of prostate cancer when they had the *p21 C98A* CA genotype compared to non-smokers with the CC genotype.

Since the *p53* codon 72 polymorphism was first identified in 1987 (14), several studies have reported the effects of the *p53* codon 72 polymorphism on prostate cancer risk within different ethnic populations. The results from those studies were controversial (24-30). In particular, the *Arg* and *Pro* alleles are associated with a high risk of malignancy. No association was identified in the present study with altered prostate cancer risk between cases and controls for the *Pro/Pro* genotype (OR=1.08; 95% CI, 0.47-2.44; P>0.05) in comparison with the *Arg/Arg* genotype. These findings are not in agreement with the original study of Henner *et al* (38) in a predominantly Caucasian population. The Henner *et al* (38) study found a protective effect of the *Pro/Pro* genotype and a significant lowering risk of prostate cancer with this genotype (OR=0.14; 95% CI, 0.03-0.71; P=0.017) in comparison to the *Arg/Arg* genotype. However, the distribution of the *p53* codon 72 genotypes violated the rule of Hardy-Weinberg equilibrium. By contrast, another study of a Caucasian population performed by Quiñones *et al* (21) identified a positive association of the *Pro/Pro* genotype with prostate cancer risk (OR=2.89; 95% CI, 1.17-7.10). Studies carried out in Japan (22), China (23) and Northern India (24) reported the same increased association of the *Pro* allele with prostate cancer risk as the study by Quiñones *et al* (21), however, the studies of men in Argentina (27) and Iran (28) did not. One study by Ricks-Santi *et al* (26) found a significant association of the *Arg* allele with the prevalence of prostate cancer in populations of men of African descent.

The first meta-analysis to be performed comprised 582 prostate cancer patients and 1,075 controls (29). The meta-analysis observed no associations of the *p53* codon 72 polymorphism with prostate cancer (for *Arg/Arg* vs. *Pro/Pro*: OR=0.88; 95% CI, 0.62-1.25; for the dominant model: OR=1.05; 95% CI, 0.78-1.43; for the recessive model: OR=0.85; 95% CI, 0.67-1.06), for the overall data. In the subgroup analysis by ethnicity, the study found that individuals carrying the *Arg* allele had an increased susceptibility to prostate cancer compared with those carrying the *Pro* allele in the Caucasian, but not Asian, population. These findings were confirmed by a subsequent meta-analysis of 8 independent studies (815 cases and 1,047 controls), which were carried out on Japanese, Chinese, American, Argentinian and Chilean populations (30). The authors concluded that the *p53* codon 72 polymorphism is not associated with prostate cancer risk and the same consistent result was identified when stratifying for the ethnicity. A subsequent meta-analysis of 17 case-control studies involving 2,371 prostate cancer patients and 2,854 controls suggested that the *Pro/Pro* genotype of the *p53* codon 72 polymorphism was associated with an increased prostate cancer risk, particularly among Caucasians (25).

The difference in the results of prostate cancer risk association in the present study, as well as other previous studies, may be explained as follows: i) The frequencies of *p53 Arg* and *Pro* alleles and haplotypes differ across ethnicities (39), which may be the leading cause for different effects of the *p53* codon 72 polymorphism on prostate cancer risk in different ethnicities. ii) Different study design, sample size, genotyping method and source of controls may be responsible for the conflicting findings among individual studies. Certain studies had reduced sample size and did not have an adequate scope to detect the likely risk for the *p53* codon 72 polymorphism. iii) The two polymorphic variants of *p53* may be involved in selectively regulating specific cellular functions, and therefore the functional differences between the 2 forms of *p53* suggest that their expression status may therefore influence the cancer

risk. As has been reported, the *Arg* allele on *p53* has a loci closer to the mitochondria. This may lead to the release of cytochrome *c* into the cytosol, which subsequently further enhances the apoptotic activity similar to that observed with the *Pro* allele. By contrast, the *Pro* form appeared to induce a higher level of G_1 arrest compared with the *Arg* form (17). The presence of *Arg* in the mutant allele or preferential retention of the *Arg* allele in the tumoral tissue provides a selective growth advantage to tumor cells during tumorigenesis (40). iv) The influence of the *p53* polymorphism may be masked by the presence of other, not yet identified, causal genes involved in prostate cancer development.

The role of the p21 protein in modulating cell cycle regulation has been well established. The p21 protein is a downstream target of p53. In response to DNA damage, increased expression of *p21* following p53 activation leads to either cell-cycle arrest at the G_1 checkpoint or apoptosis. Expression can suppress tumor growth through inhibition of proliferating cell nuclear antigen-dependent DNA replication and mismatch repair *in vitro* (41). In addition, the overexpression of *p21* may prevent mammalian cell proliferation and inhibit all cyclin-CDK complexes, suggesting that p21 is a universal inhibitor of cyclin-CDK complexes (42). Furthermore, overexpression of *p21* and the subsequent overall reduced CDK activity is associated with cell differentiation (43). Mutations of *p21* are extremely rare and the SNPs are more likely to have a functional effect in cancer. In the present study, no association between the *p21 C98A* polymorphism and prostate cancer risk was found as a result of a non-significant protective effect of the *p21 C98A* CA polymorphic variant on prostate cancer risk (OR=0.73; 95% CI, 0.50-1.06; P>0.05). A few previous studies reported a significant role of the *p21 C98A* polymorphism in the development of prostate cancer (35,44,45). The *p21 C98A* polymorphism causes a Ser-to-Arg substitution in its zinc-finger motif, which could alter the protein function of p21 (33). By contrast, *in vitro* transfection studies suggest the *Arg* allele of this variant has a similar functional activity to the wild-type *Ser* allele (46).

The *p21 C70T* polymorphism at exon 3 (which lies within the 3' UTR 20 nt downstream of the stop codon) has been hypothesized to possibly increase the cancer risk by altering mRNA stability, thereby affecting intracellular levels of the p21 protein (34). However, the present study found no significant association between this polymorphism and prostate cancer risk (OR=1.06; 95% CI, 0.72-1.58; P>0.05), as was also previously reported in our relatively small pilot study (47). Only one published case-control study evaluated the association of the *p21 C70T* polymorphic genotypes CT and TT with the risk of advanced prostate carcinoma in a European-American population (OR=2.24; 95% CI, 1.02-4.95) and these genotypes were more strongly associated with more aggressive metastatic disease (androgen-independent disease or fatality from metastatic prostate carcinoma) (48). These data may not exclude the possibility that p21 may have a role in prostate cancer and this hypothesis remains to be evaluated in future studies.

In the p53 pathway, p53 and p21 have a crucial role together; the p21 protein regulates the abundance, subcellular localization and transcriptional function of p53. Based on this evidence, whether or not these gene polymorphisms and their gene-gene interaction (*p53-p21*) may be important in the development of prostate cancer was investigated. The result suggests that the *p53* codon 72 (*Arg*/*Pro*+*Pro*/*Pro* genotype) and *p21 C98A* (*CA* genotype) polymorphisms are likely to synergistically affect the events leading up to the development of prostate cancer (OR=0.49; 95% CI, 0.27-0.86; P<0.05). Additionally, patients with *p53* codon 72 *Arg*/*Pro*+*Pro*/*Pro* and *p21 C70T* CC and CT genotypes did not show a significant decrease of prostate cancer risk. Therefore, we hypothesize that the lower apoptotic rate induced by the *Pro* allele combined with the *p21 C98A* polymorphism that alter the protein function of p21, thereby influencing the cellular DNA damage-induced cell cycle arrest response, may decrease the risk of prostate cancer. Although it is unclear from these results which of the functional differences between these polymorphic alleles is more important, it would be noteworthy to investigate further the molecular mechanism of the p53/p21-mediated cell cycle arrest in the development of prostate cancer.

No significant correlation was identified between these 3 polymorphisms with serum PSA levels and Gleason score. Additionally, the studies of Huang *et al* (49) and Sun *et al* (50) did not find an association between the *p53* codon 72 polymorphism and the clinicopathological features or recurrence of PSA for clinical localized prostate cancer following radical prostatectomy. Huang *et al* (49) reported the effect of the *p21 C98A* AA genotype on the prostate cancer risk to be significant for localized disease and significant for locally advanced disease. When stratified by pathological grade, the *p21 C98A* AA genotype was also found to be associated with the significantly increased risk for moderately differentiated prostate cancer (OR=2.04; 95% CI, 1.17-3.53), whereas the *p21 C98A* AA genotype was not associated with either poorly or well-differentiated prostate cancer. To the best of our knowledge, there is no study thus far that evaluates the effect of the *p21 C70T* polymorphisms on the serum PSA levels and Gleason score in prostate cancer patients. Thus, we speculate that the difference observed in the results of these studies may be due to a multistep process with numerous factors contributing to its pathogenesis and progression. These 3 polymorphisms may not influence later events as other factors may begin to have a greater influence for tumorigenesis.

Prostate cancer risk increases with cigarette smoking and other environmental exposures. While the molecular mechanisms of the tobacco smoke association with carcinogenesis remain unclear, a polycyclic aromatic hydrocarbon and potent carcinogen, benzopyrene, activates the epidermal growth factor receptor and cell proliferation (51). Thus, in the present study the possibility of an association of *p53* codon 72, *p21 C98A* and *p21 C70T* polymorphisms and tobacco smoke in prostate cancer development was analyzed. This decision was also influenced by the fact that no study has as yet reported an association of the *p21 C98A* and *p21 C70T* polymorphisms with prostate cancer risk and smoking status. When considering the stratified analysis based on the smoking status, smokers with the *Pro* allele had an ~2.97-fold increased prostate cancer associated risk compared with those with the wild-type genotype (*Arg*/*Arg*). This finding disagrees with the earlier observation of Mittal *et al* (24), which found no association of the *p53* codon 72 polymorphism and the use of tobacco with prostate cancer risk. Of note, in non-smokers the *p21 C98A* CA genotype had a statistically

significant protective role in prostate cancer risk (OR=0.58; 95% CI, 0.36-0.93; P<0.05).

In conclusion, the present case-control study indicates that individually the *p53* and *p21* polymorphisms may not be associated with an increased risk of prostate cancer within the Slovak population. By contrast, subjects with the combined genotypes *p53* codon 72 *Arg/Pro+Pro/Pro* and *p21 C98A* CA had a significantly lower prostate cancer risk compared with those with the combined reference *p53* codon 72 *Arg/Arg* and *p21 C98A* CC genotypes. Therefore, the potential effect of gene-gene and gene-environment interactions on prostate cancer development requires further investigation in future studies with larger, multiethnic populations to elucidate the underlying mechanism that may link these *p53* and *p21* polymorphisms to prostate cancer risk.

Acknowledgements

The present study was supported by the Ministry of Health of the Slovak Republic under the project 2012/27-UKMA-4 'Study of molecular changes of selected genes and their potential role as a diagnostic and prognostic markers of prostate cancer' and by project 'Center of Excellence for Research in Personalized Therapy,' code 2622012053, co-financed from EU sources and the European Regional Development Fund. The authors are grateful to Dr N.A. Yeboah for the critical reading of the manuscript and wish to thank Mrs. M. Martinčeková for her technical assistance.

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