Tumor Biol.
DOI 10.1007/s13277-014-2604-7

RESEARCH ARTICLE

Detection of human papillomavirus (HPV) DNA prevalence and p53 codon 72 (Arg72Pro) polymorphism in prostate cancer in a Greek group of patients

Vasiliki Michopoulou · Stavros P. Derdas · Emmanouil Symvoulakis · Nikolaos Mourmouras · Alexandros Nomikos · Dimitris Delakas · George Sourvinos · Demetrios A. Spandidos

Received: 21 June 2014 / Accepted: 4 September 2014

Abstract Prostate cancer is the most common neoplasm found in males and the second most frequent cause of cancer-related mortality in males in Greece. Among other pathogens, the detection frequency of human papillomavirus (HPV) has been found to be significantly increased in tumor tissues among patients with sexually transmitted diseases (STDs), depending on the geographical distribution of each population studied. The present study focused on the detection of HPV and the distribution of Arg72Pro p53 polymorphism in a cohort of healthy individuals, as well as prostate cancer patients. We investigated the presence of HPV in 50 paraffin-embedded prostate cancer tissues, as well as in 30 physiological tissue samples from healthy individuals by real-time PCR. Furthermore, the same group of patients was also screened for the presence of the Arg72Pro polymorphism of the p53 gene, a p53 polymorphism related to HPV. Out of the 30 control samples, only 1 was found positive for HPV (3.33 %). On the contrary, HPV DNA was detected in 8 out of the total 50 samples (16 %) in the prostate cancer samples. The distribution of the three genotypes, Arg/Arg, Arg/Pro, and Pro/Pro, was 69.6, 21.7, and 8.7 % in the cancer patients and 75.0, 17.86, and 7.14 % in healthy controls, respectively. No statistically significant association was observed between the HPV presence and the age, stage, p53 polymorphism status at codon 72, or PSA. The increased prevalence of HPV detected in the prostate cancer tissues is in agreement with that reported in previous studies, further supporting the association of HPV infection and prostate cancer.

Keywords Prostate cancer · HPV · p53 codon 72 polymorphism · Prostate cancer patients

V. Michopoulou · S. P. Derdas · E. Symvoulakis · G. Sourvinos (✉) · D. A. Spandidos
Laboratory of Virology, Faculty of Medicine, University of Crete, Heraklion 71003, Crete, Greece
e-mail: sourvino@med.uoc.gr

N. Mourmouras · A. Nomikos · D. Delakas
Department of Urology, Asklipieio General Hospital, Athens, Greece

Introduction

It is known that the most common male malignancy worldwide is prostate cancer [1]. Among the prostate malignancies, adenocarcinoma, which is a carcinoma of the glandular tissue, is the most frequent [2]. Apart from adenocarcinoma [3], other types of cancer, although rare, can also initiate from the prostate gland, including sarcomas, small cell carcinomas, and transitional cell carcinomas [4, 5]. Although, the etiology of prostate cancer is largely known, there are several risk factors that are considered to contribute toward the development of this disease. These include hormonal disorders, advanced age, African-American race, and family history [6]. Other risk factors, such as diet, physical activity, and occupational exposures, have also been implicated; however, studies have demonstrated conflicting results [7, 8].

Historically, Ravich and Ravich [9] first proposed in 1951 that sexually transmitted infections (STIs) may also contribute to prostate carcinogenesis. Since then, there have been several epidemiological studies that have focused on the association between STDs and prostate cancer [10–15]. STDs are generally known to increase the risk of prostate cancer by causing inflammation of the prostate, which may then lead to the initiation of carcinogenesis [7, 8]. However, the association of other STIs, such as *Chlamydia trachomatis*, human papilloma virus (HPV), and human herpesvirus type 8 (HHV-8)

Published online: 12 September 2014

infections, and prostate cancer development is controversial [16–18].

Human papilloma virus is the most common STI worldwide according to the Center for Disease Control and Prevention (CDC). However, the experimental data and epidemiological estimates in the male population differ among several studies. Differences in HPV prevalence in sexually active men vary depending on the geographical area, the anatomical site, as well as the sampling method. High-risk groups comprise of men with multiple sex partners and men infected with human immunodeficiency virus (HIV) [19, 20]. The HPV detection in these groups has considerable variation, ranging from 1 to 84 % among the low-risk group and from 2 to 93 % among the high-risk group [21]. Several studies have focused on HPV detection using molecular diagnostic techniques and possible correlation with prostate cancer with controversial results [22–25].

Human papillomavirus detection was initially carried out using in situ hybridization, as well as direct probe hybridization, such as dot blot, and Northern or Southern blots [26]. However, these techniques require a significant amount of viral DNA to be present in the sample, while they are generally quite laborious and time-consuming. The immunological detection of HPV is not regarded to be reliable, mainly due to three reasons. Firstly, the capsid proteins are only expressed in active infection; secondly, the early viral proteins are expressed in low amounts and, finally, the available antibodies lack the sensitivity and affinity for the viral proteins [27]. These abovementioned techniques have been superseded by the molecular detection of HPV DNA using polymerase chain reaction (PCR), as well real-time PCR [28]. Both these techniques provide increased sensitivity, detecting a low-copy number of viral particles, as well as the specific high-risk subtypes of HPV, while they can be performed in a relatively short time period.

p53 is a 53-kDa nuclear phosphoprotein consisting of 393 amino acids[29], and is known as protein 53 or tumor protein 53 and in humans is encoded by the TP53 gene, located on the short arm of chromosome 17 (17p13.1) [30]. This protein plays a crucial role in organism since it regulates the cell cycle and, thus, functions as a tumor suppressor that is involved in preventing cancer [31]. p53 has been described as " the guardian of the genome" since it protects the cell by preventing genome mutation. Countless publications had shown that p53 mutations are found in the majority of human tumors, while in the control groups of the surveys, no mutations in p53 were observed [32, 33]. As for the ten most frequent cancer types, p53 alterations are observed in 40–45 % of cases [34].

The p53 gene polymorphism is a common human polymorphism concerning codon 72 substitution of the amino acid of arginine (Arg) for a proline (Pro). Many studies have investigated the correlation between this polymorphism and cancer development with controversial results. p53 protein with Arg (p53-Arg72) seems to be more susceptible to E6-mediated degradation than the Pro form (p53-Pro72), and according to several reports, the Arg allele is overrepresented in cancer patients [35, 36]. As for prostate cancer, there are few studies on the effect of the Arg polymorphism and increased risk for cancer development, with controversial results [23, 37–39]. On the other hand, there are studies supporting that Pro homozygosity is associated with a reduced risk of prostate cancer [38], and therefore this allele may have some protective effects.

The aim of this study was to examine the presence of human papilloma virus in prostate cancer patients and healthy individuals, using real-time PCR. Subsequently, we examined the genotypic frequency of the p53 codon 72 polymorphism in the same cohort of patients. The final goal was to identify any possible correlation between HPV infection, the p53 polymorphism, and a higher risk of developing prostate cancer. In addition, the results of this study were associated with the patient histopathological status, their age, and the value of the prostate-specific antigen (PSA), as well as other studies focusing on HPV detection, p53 codon 72 polymorphism, and prostate cancer risk.

Materials and methods

Tumor specimens

Fifty tumor specimens were acquired from patients with histologically confirmed prostate cancer, as well as 30 samples from healthy individuals from the Department of Urology, "Asklipieio" General Hospital, Voula, Athens, Greece. The tissues were formalin-fixed and embedded in paraffin blocks after surgical removal. All samples were randomly selected from the archival collection of the Department of Urology. The ethics committee of the "Asklipieio" General Hospital approved the protocol and all patients and control subjects provided informed consent in written form.

DNA isolation

Five 20-μm-thick sections were cut from each block and placed in 2-ml sterile tubes. Tissues were deparaffinized by immersing in xylene for 10 min at 60 °C, then centrifuged at 10,000 rpm at room temperature (this step was performed twice). Finally, each sample was washed with absolute ethanol three times (10,000 rpm at 4 °C for 5 min) and then dried at 37 °C. To extract DNA, the samples were incubated in 400-μl Lysis buffer (10 mM Tris, 0.1 mM EDTA, and 2 % SDS), using 30 μl Proteinase K (10 mg/ml) at 55 °C and at 700 rpm using a shaking incubator for 48 h. The samples were supplemented with Proteinase K after the first 24 h.

DNA extraction was performed according to the Phenol/Chloroform protocol. DNA was precipitated by using absolute ethanol at −20 °C overnight. The amount of DNA was determined by ultraviolet (UV) absorbance at 260 nm and brought to a final concentration of 100 ng/μl.

Polymerase chain reaction for β-actin gene detection

Polymerase chain reaction (PCR) was performed for all 80 samples, to detect amplifiable DNA, using primers for the β-actin gene (Table 1). The PCR conditions for the amplification of the β-actin gene were set in a Thermocycler machine according to the following:

An initial denaturation step at 94 °C for 4 min and then 36 cycles of 94 °C for 30 s, annealing at 58 °C for 30 s, and the elongation step at 72 °C for 30 s. The final elongation step of the PCR was carried out at 72 °C for 10 min.

Real-time polymerase chain reaction for HPV detection

Real-time polymerase chain reaction (RT-PCR) was performed for all 80 samples that produced amplifiable DNA in the β-actin PCR. The widely accepted $GP5^{+}/6^{+}$ primer set (Table 1) was used for the identification of HPV-positive samples [40, 41]. Real-time PCR was carried out in an Applied Biosystems (AB-7500) RT-PCR Detection System (Applied Biosystems, Carlsbad, CA, USA). Positive, as well as negative (DNA-free), samples were included in each run. All reactions were performed in a final volume of 20 μl containing 2× Kappa SYBR Green Fast qPCR™, 50× ROX Low reference dye, 50 pmol $GP5^{+}/6^{+}$ primers, and 1 μl DNA (100 ng/μl). The amplification protocol for the HPV-DNA detection was optimized prior to the study sample testing. The annealing temperature was selected in the range between 38 and 50 °C by automatic gradient analysis on sequenced HPV positive samples [42–44].

In order to verify the correct discrimination between HPV-positive, -negative, and primer-dimer products, gel electrophoresis was performed onto a 2 % agarose gel. Apparently at 44 °C, the product had the best yield and target discrimination, producing the specific product common among all HPV subtypes. Therefore, we used the following amplification protocol for HPV-DNA analysis: initial denaturation at 95 °C for 3 min, and then 40 cycles of 95 °C for 30 s, 44 °C for 1 min, and 72 °C for 1:30 min. A final elongation step was added at 72 °C for 10 min. After amplification, a melting curve was performed at temperatures between 60 and 95 °C with temperature increasing at 0.5 °C per second. The melting temperature (*T*m) range to distinguish the $GP5^{+}/6^{+}$ amplicons was between 77 and 82 °C.

Table 1 Primer sequences and length of PCR products

Primers	Sequence (5′–3′)	PCR product (bp)
β-actin F	CGGCATCGTCACCAACTG	70
β-actin R	GGCACACGCAGCTCATTG	
GP5	TTT GTT ACT GTG GTA GAT AC	150
GP6	GAA AAA TAA ACT GTA AAT CA	
ArgF	TCC CCC TTG CCG TCC CAA	141
ArgR	CTG GTG CAG GGG CCA CGC	
ProF	GCC AGA GGC TGC TCC CCC	177
ProR	CGT GCA AGT CAC AGA CTT	

Polymerase chain reaction amplification of p53 polymorphic sequences

For the determination of the polymorphism at codon 72 of the p53 gene, two sets of primers were used, one to amplify the Arg allele and the other to amplify the Pro allele (Table 1). PCR was performed for all 80 samples, twice, one time for each allele. The PCR was transacted in a Thermocycler machine according the following conditions. For the Arg allele, there was an initial denaturation step at 94 °C for 3 min and then 35 cycles of 94 °C for 30 s, annealing at 60 °C for 30 s, and the elongation step at 72 °C for 30 s. The final elongation step of the PCR was carried out at 72 °C for 5 min. As for the Pro allele, the same program was followed, differentiated at the annealing step which was at 54 °C for 30 s. All reactions were performed in a final volume of 25 μl containing 1× Mg free Taq buffer, 25 mM MgCl2, 5 mM of each dNTP, 10 mM of each primer (either ArgR/ArgF set or ProR/ProF set), 0.5 U/ml Taq polymerase (KAPA Taq PCR Kit, KAPA BIOSYSTEMS), and 1 μl DNA (100 ng/μl).

PCR product analysis

The PCR products were electrophoresed onto a 1.5 % agarose gel, stained with Thermo Scientific 6× DNA loading dye, and then photographed on a UV light transilluminator. The photographs were processed using Adobe Photoshop CS3 Extended for Windows. The PCR product of the Arg allele was 141 bp, while the product of the Pro allele was 177 bp. Heterozygous specimens had PCR products in both PCRs, whereas homozygous samples exhibited product only in one of the two reactions. In each PCR reaction, there were two blank samples as negative controls, to ensure that no contaminants were introduced.

HPV genotyping

All HPV-positive samples were subjected to genotyping. The LINEAR ARRAY HPV Genotyping (Roche) Test, which is a qualitative test that detects 37 high- and low-risk human papillomavirus genotypes, was employed according to the

manufacturer's instructions. This kit was selected for its high sensitivity, specificity, and reliability [45].

Statistical analysis

Continuous variables are presented as the means±standard deviation [age, prostate-specific antigen (PSA)], while categorical data (age groups, tumor stage, and p53 polymorphism at codon 72) are described with the use of counts and frequencies. Pearson's chi-square test and Fisher's exact test, due to the small sample sizes, were used to test possible differences in regards to HPV presence between prostate cancer cases and controls. The analysis was performed with the statistical package SPSS 16.0 (Statistical Package for Social Sciences, SPSS, Inc, Illinois, USA). *P* values were based on two-sided tests and a significance level lower than 0.05 was adopted.

Results

Thirty-eight patients with prostate cancer were diagnosed at stage pT2 (76 %), 7 at stage pT3 (14 %), and 5 at stage pT4 (10 %). The mean age of the patients was 65.5 (SD 5.97) years. Among the 35 cancer cases with available data, the mean value of prostate-specific antigen (PSA) was 9.19 ng/ml (SD 9.12; Table 2). Thirty-six tumors (78.3 %) were found at "homozygotic" status within the p53 polymorphism 72 Arg or Pro, while 10 tumors (21.7 %) were found at "heterozygotic" status presenting p53 polymorphism at codon 72 Arg and Pro (4 missing cases). Respectively, 23 controls (82.1 %) were either Arg/Arg or Pro/Pro, while 5 healthy samples (17.9 %) followed the Arg/Pro model as for p53 polymorphism 72 (2 missing cases). The distribution of p53 alleles codon 72 Arg or Pro is presented in Fig. 1, while representative examples of the detection of the PCR analysis are shown in Fig. 2.

Analysis for the detection of HPV virus revealed 8 positive samples out of 50 (16 %) prostate tumor specimens. Four samples were infected by HPV-18, two by HPV-16, one by HPV-31, and for one sample the HPV type could not be determined by the assay employed. On the contrary, only 1 out of 30 controls (3.4 %) was found positive for HPV (undetermined type). Statistical analysis between the HPV-positive prostate cancer samples [8/50 (16 %)] and control specimens [1/30 (3.4 %)] produced a value of $P=0.086$ by performing Pearson's chi-square test and $P=0.080$ with Fisher's exact test (Fig. 3). Although this difference met a borderline level of nonstatistical significance, an attempt to calculate the respective odds ratio (OR) led to a 5.52 (confidence interval 95 % 0.66–45.6). HPV infection was detected in 6/38 (15.8 %) samples from patients at stage II, in 0/7 (0 %) samples from patients at stage III, and in 2/5 (40 %) samples from patients at stage IV. Table 3 shows the frequencies of HPV DNA in the age groups of prostate cancer patients < or ≥70 years. Overall, we did not observe any statistically significant association of HPV presence with age, stage, p53 polymorphism status at codon 72, and PSA.

Discussion

According to the American Cancer Society projections, in 2013, there were 580,350 cancer deaths overall. Among them, 306,920 were male deaths, including 29,720 from prostate cancer [46]. Prostate cancer is the most common cancer type after skin cancer and the second leading cause of cancer-related mortality, following lung carcinomas [47]. For 2014, the American Cancer Society estimated that approximately 233,000 new cases of prostate cancer will be diagnosed and approximately 29,480 men will die of prostate cancer in the USA. The overall prediction for the male population is that 1 in 7 men will be diagnosed with prostate cancer during his lifetime, while approximately 1 in 36 men will die of prostate cancer [48].

Several studies have focused on HPV detection using molecular diagnostic techniques and its correlation with prostate cancer with controversial results. Specifically, HPV DNA was detected in 20 % of prostate cancer specimens and in 3 % of the control group in a recent study [22]. Concomitantly, another study demonstrated an increased association between HPV DNA detection and prostate adenocarcinoma with 41.5 % prevalence of HPV in prostate cancer cases [23]. On the contrary, two independent studies reported no detection of HPV DNA in 352 and 200 prostate tissues samples screened [24, 25]. The differences observed in the abovementioned studies may be justified by differences in the study populations (differences in sexual behavior and thus exposure to HPV), as well as technical differences in the viral detection methods.

In 1998, the fundamental study of Storey et al. [35] described the role of the p53 polymorphism in the development of HPV-associated cancer, stating that the HPV E6 oncoprotein binds p53 and promotes its ubiquitin-mediated degradation [49, 50] with the Arg form to be more susceptible rather than the Pro form. As a consequence, individuals homozygous for Arg were found to be seven times more susceptible to HPV-associated tumorigenesis than heterozygotes. Since then, many studies have investigated the correlation between the p53 polymorphism and several types of cancer, such as cervical [51], pancreatic [52], breast [53], colorectal [54], endometrial [55], renal cell carcinoma [56], nonoropharyngeal [57], lung [58], urinary bladder [36], and prostate cancer [23, 37–39] development with controversial results.

Concerning prostate cancer, Henner et al. [38] suggested that men with p53 homozygosity for Pro seem to be at a

Table 2 Clinical and histopathological data of 50 patients with prostate cancer and results of p53 codon 72 polymorphism analysis and HPV detection

No	Arg/Arg	Arg/Pro	Pro/Pro	HPV	HPV type	TNM	Age	PSA (ng/ml)
1		+				T2cNoMo	71	50.70
2	+			+	18	T2cNoMo	52	NA
3	+					T2cN1Mo	66	9.2
4	+					T2cNoMo	60	NA
5	+			+	16	T2cNoMo	73	5.08
6			+			T2cNoMo	66	34.61
7	+					T2cNoMo	73	9.32
8		+				T3bNoMo	66	12.5
9		+				T2cNoMo	62	NA
10	+					T2cNoMo	73	1.98
11	+					T3bNoMo	72	8.7
12	+					T2cNoMo	55	5.8
13		+				T2cNoMo	74	7.76
14		+		+	16	T2cNoMo	68	4.8
15	+					T2cNoMo	74	7.91
16	+					T2bNoMo	62	8.46
17						T2cNoMo	66	NA
18						T3bNoM1b	67	NA
19		+				T2bNoMo	74	NA
20	+					T3aNoMo	65	2.8
21		+		+	18	T2cNoM1bM1c	71	7.0
22	+					T2cNoMo	68	8.9
23	+					T2bNoMo	71	5.0
24		+				T2bNoMo	65	10.09
25			+			T2cNoMo	64	13
26		+				T2cNoMo	59	6.14
27	+					T2cNoMo	70	5.3
28	+					T2cNoMo	61	4.3
29		+				T2bNoMo	59	6.1
30	+			+	18	T2bNoMo	59	8.8
31	+					T2cNoMo	68	4.4
32	+					T3bNoMo	70	NA
33	+					T2bNoMo	65	3.8
34	+					T2cNoMo	59	4.8
35			+	+	31	T2cNoMo	58	NA
36						T2bNoMo	71	8.5
37	+			+	Undefined	T2cNoM1b	50	6.52
38	+					T2cNoMo	68	9.0
39	+					T2cNoM1b	67	4.70
40	+					T3bNoMo	68	18.1
41	+					T2cNoMo	65	NA
42						T2cNoMo	60	NA
43	+					T2cNoMo	59	NA
44	+					T2cNoMo	70	NA
45	+			+	18	T2cNoMo	67	NA
46	+					T3bNoMo	74	5.6
47	+					T2cNoMo	68	NA
48	+					T2cNoMo	67	NA
49	+					T2bNoMo	58	5.84
50	+					T2bNoMo	58	6.3

TNM staging: *T* evaluation of the primary tumor, *N* evaluation of the regional lymph nodes, *M* evaluation of distant metastasis

NA not available

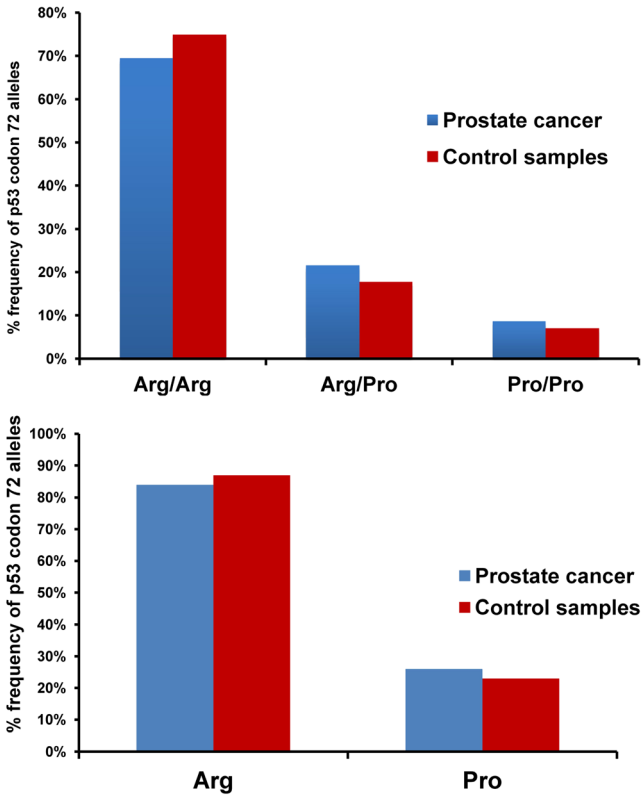

Fig. 1 p53 codon 72 polymorphism PCR analysis of both prostate cancer and control samples. **a** Distribution of proline and arginine homozygosity and heterozygosity in prostate cancer and normal samples. **b** Frequency of proline and arginine alleles in prostate cancer and normal samples

reduced risk of developing prostate cancer and therefore this allele may have some protective effect. On the contrary, three other studies found no correlation between the p53 polymorphism and prostate cancer. In detail, Wu et al. [39], in 1995 studying this polymorphism in several urological cancers, found no significant difference between the tumor and control samples and concluded that the association is not so clear. Some years later, Huang et al. [37] not only supported that

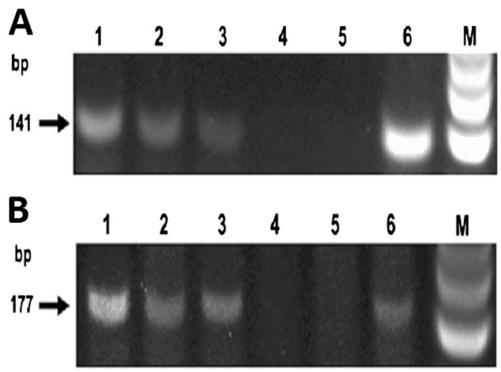

Fig. 2 **a** PCR amplification of the p53 codon 72 Arg allele (141 bp). *Lanes 1*, *2*, *3*, and *6* positive samples. *Lanes 4* and *5* negative samples. *M* 100 bp molecular weight marker. **b** PCR amplification of the p53 codon 72 Pro allele (177 bp). *Lanes 1*, *2*, *3*, and *6* positive samples. *Lanes 4* and *5* negative samples. *M* 100 bp molecular weight marker

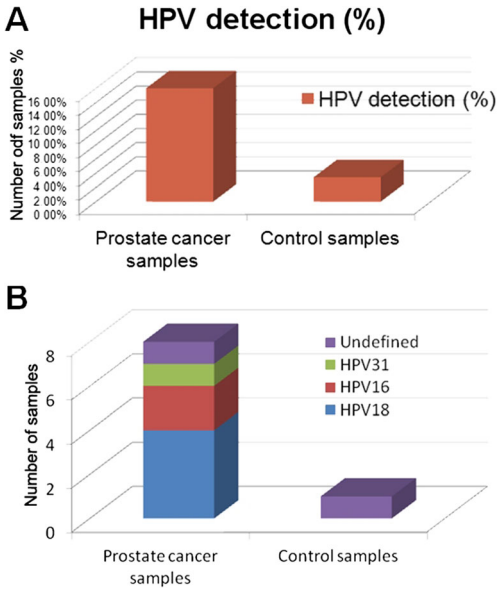

Fig. 3 HPV detection in cancer and control prostate samples. **a** Prevalence of HPV detection by real-time PCR; $P=0.086$ (Pearson's chi-square test) and $P=0.080$ (Fisher's exact test). **b** HPV genotyping results using LINEAR ARRAY HPV Genotyping (Roche) Test

there is no correlation between the p53 polymorphism and prostate cancer, but also introduced the theory that p21 codon 31 polymorphism is associated both with cancer development and benign prostate hyperplasia (BPH). More recently, Leiros et al. [23] corroborated with the above concept, concluding that the p53 codon 72 polymorphism seems to have a correlation neither with prostate carcinomas and hyperplasias nor with HPV-positive and HPV-negative carcinomas.

The present study investigated the potential association of HPV and prostate cancer using real-time PCR in archived tissue specimens from 50 cancer cases and 30 controls in a Greek group of patients. Furthermore, we examined the prevalence of p53 codon 72 polymorphism to our samples, both prostate cancer and controls and tried to associate all of the above with the demographic data, such as the age of patients, as well as tumor stage and PSA. Due to the weakness of the techniques employed in previous studies that may have contributed to reported controversial results of this association,

Table 3 HPV infection and patients with prostate cancer < or ≥70 years

Age	HPV testing	
	Positive *n* (%)	Negative *n* (%)
<70 years	6 (15.8)	32 (84.2)
≥70 years	2 (16.6)	10 (83.4)
P	NS	

HPV+ HPV-positive samples, *HPV−* HPV-negative samples
NS not significant

we selected real-time PCR as a superior and more sensitive technique.

Despite the high sensitivity and specificity of real-time PCR as a laboratory method for HPV detection in prostate cancer, we found a nonstatistically significant association between the above, apart from a trend that more patients with prostate cancer were positive for HPV infection (16 %) versus the control samples (3.4 %). HPV typing revealed that the majority of the tumor samples harbored HPV-18 (four samples), compared to HPV-16 (two samples) and other or undefined types (two samples). The high prevalence of this HPV type in the Greek population may be attributed to ethnic variations [59]. As for the p53 polymorphism, there has been a prevalence of the Arg allele in both cancer and control samples, presenting no statistically significant diversification among them. Consecutively, our results do not follow the mechanism proposed by Storey et al. [35] and agree with the abovementioned studies, supporting that there is no correlation between the p53 polymorphism, HPV infection, and prostate cancer. At last, we failed to detect any statistically significant association between HPV and the cancer stage, the age of the patients, and the p53 polymorphism status at codon 72 or PSA.

Several factors may have contributed to these findings. We used archived specimens for this study and perhaps fresh tissue may have demonstrated different results, since fresh-frozen tissue ensures higher quality in DNA isolation and PCR amplification. Furthermore, there is the risk of overestimating p53 homozygosity in tumor versus blood samples; therefore, there are reports suggesting that the genotype be conducted on blood samples [60]. The patient population from which the tissue originated may be the reason for the lack of significant difference in the presence of HPV between cancer and controls. Our population was an outcome of simple random sampling. That may lead to a sample with high Arg frequency in which a further increase in tumor patients may not yield statistically significant results versus samples comprised of higher Pro frequency [61]. Moreover, we did not have information on social or sexual behaviors of the patients in the cancer group nor the controls, an important piece of information. Considering that HPV presence is strongly associated with high-risk sexual behavior, a comparison of cancers from patients with such sexual activity versus those who did not engage in high-risk behavior may have shown different results. Lastly, the lack of a statistical association between HPV infection and p53 codon 72 Arg homozygosity indicates a synergistic action of the virus with other environmental factors, such as exposure to carcinogens or other lifestyle habits leading to the development of the disease, information that we do not have. Last but not least, another handicap of our research may be the sample size. Considering the trend that seems to show high, regarding HPV prevalence in prostate cancer versus control samples, a broader sample may have displayed statistically significant results. Although with the currently available superior laboratory techniques HPV presence in prostate cancer can be easily demonstrated, the true association, if any, of the virus with the disease requires further investigation.

Conflicts of interest None

References

1. Stanford JL, Just JJ, Gibbs M, Wicklund KG, Neal CL, Blumenstein BA, et al. Polymorphic repeats in the androgen receptor gene: molecular markers of prostate cancer risk. Cancer Res. 1997;57:1194–8.
2. Ketelsen D, Rothke M, Aschoff P, Merseburger AS, Lichy MP, Reimold M, et al. Detection of bone metastasis of prostate cancer—comparison of whole-body MRI and bone scintigraphy. Röfo. 2008;180:746–52.
3. CDC. Prostate cancer: the public health perspective, Available at www.cdc.gov/cancer. 2012.
4. Taylor ML, Mainous 3rd AG, Wells BJ. Prostate cancer and sexually transmitted diseases: a meta-analysis. Fam Med. 2005;37:506–12.
5. Lee CH, Akin-Olugbade O, Kirschenbaum A. Overview of prostate anatomy, histology, and pathology. Endocrinol Metab Clin N Am. 2011;40:565–75. viii-ix.
6. Hoffman RM. Clinical practice. Screening for prostate cancer. N Engl J Med. 2011;365:2013–9.
7. Dennis LK, Dawson DV. Meta-analysis of measures of sexual activity and prostate cancer. Epidemiology. 2002;13:72–9.
8. Canby-Hagino ED, Thompson IM. Mechanisms of disease: prostate cancer—a model for cancer chemoprevention in clinical practice. Nat Clin Pract Oncol. 2005;2:255–61.
9. Ravich A, Ravich RA. Prophylaxis of cancer of the prostate, penis, and cervix by circumcision. NY State J Med. 1951;51:1519–20.
10. Miles DD. Cancer of the prostate: The silent man-killer. Del Med J. 1970;42:1–3. passim.
11. Nomura AM, Kolonel LN. Prostate cancer: a current perspective. Epidemiol Rev. 1991;13:200–27.
12. Pienta KJ, Esper PS. Risk factors for prostate cancer. Ann Intern Med. 1993;118:793–803.
13. Wideroff L, Schottenfeld D, Carey TE, Beals T, Fu G, Sakr W, et al. Human papillomavirus DNA in malignant and hyperplastic prostate tissue of black and white males. Prostate. 1996;28:117–23.
14. Rosenblatt KA, Wicklund KG, Stanford JL. Sexual factors and the risk of prostate cancer. Am J Epidemiol. 2001;153:1152–8.
15. Sutcliffe S, Viscidi RP, Till C, Goodman PJ, Hoque AM, Hsing AW, et al. Human papillomavirus types 16, 18, and 31 serostatus and prostate cancer risk in the prostate cancer prevention trial. Cancer Epidemiol Biomarkers Prev. 2010;19:614–8.
16. Adami HO, Kuper H, Andersson SO, Bergstrom R, Dillner J. Prostate cancer risk and serologic evidence of human papilloma virus infection: a population-based case–control study. Cancer Epidemiol Biomarkers Prev. 2003;12:872–5.
17. Dillner J, Knekt P, Boman J, Lehtinen M, Af Geijersstam V, Sapp M, et al. Sero-epidemiological association between human-papillomavirus infection and risk of prostate cancer. Int J Cancer. 1998;75:564–7.
18. Hayes RB, Pottern LM, Strickler H, Rabkin C, Pope V, Swanson GM, et al. Sexual behaviour, STDs and risks for prostate cancer. Br J Cancer. 2000;82:718–25.
19. Palefsky JM, Holly EA, Ralston ML, Jay N. Prevalence and risk factors for human papillomavirus infection of the anal canal in human

immunodeficiency virus (HIV)-positive and HIV-negative homosexual men. J Infect Dis. 1998;177:361–7.
20. Partridge JM, Hughes JP, Feng Q, Winer RL, Weaver BA, Xi LF, et al. Genital human papillomavirus infection in men: incidence and risk factors in a cohort of university students. J Infect Dis. 2007;196: 1128–36.
21. Smith JS, Gilbert PA, Melendy A, Rana RK, Pimenta JM. Age-specific prevalence of human papillomavirus infection in males: a global review. J Adolesc Health. 2011;48:540–52.
22. Martinez-Fierro ML, Leach RJ, Gomez-Guerra LS, Garza-Guajardo R, Johnson-Pais T, Beuten J, et al. Identification of viral infections in the prostate and evaluation of their association with cancer. BMC Cancer. 2010;10:326.
23. Leiros GJ, Galliano SR, Sember ME, Kahn T, Schwarz E, Eiguchi K. Detection of human papillomavirus DNA and p53 codon 72 polymorphism in prostate carcinomas of patients from Argentina. BMC Urol. 2005;5:15.
24. Bergh J, Marklund I, Gustavsson C, Wiklund F, Gronberg H, Allard A, et al. No link between viral findings in the prostate and subsequent cancer development. Br J Cancer. 2007;96:137–9.
25. Sfanos KS, Sauvageot J, Fedor HL, Dick JD, De Marzo AM, Isaacs WB. A molecular analysis of prokaryotic and viral DNA sequences in prostate tissue from patients with prostate cancer indicates the presence of multiple and diverse microorganisms. Prostate. 2008;68:306–20.
26. Begum S, Gillison ML, Ansari-Lari MA, Shah K, Westra WH. Detection of human papillomavirus in cervical lymph nodes: a highly effective strategy for localizing site of tumor origin. Clin Cancer Res. 2003;9:6469–75.
27. Iftner T, Villa LL. Chapter 12: Human papillomavirus technologies. J Natl Cancer Inst Monogr. 2003;31:80–88.
28. Gravitt PE, Jamshidi R. Diagnosis and management of oncogenic cervical human papillomavirus infection. Infect Dis Clin N Am. 2005;19:439–58.
29. Burns TF, El-Deiry WS. The p53 pathway and apoptosis. J Cell Physiol. 1999;181:231–9.
30. Isobe M, Emanuel BS, Givol D, Oren M, Croce CM. Localization of gene for human p53 tumour antigen to band 17p13. Nature. 1986;320:84–5.
31. Matlashewski G, Lamb P, Pim D, Peacock J, Crawford L, Benchimol S. Isolation and characterization of a human p53 cDNA clone: expression of the human p53 gene. EMBO J. 1984;3:3257–62.
32. Caron de Fromentel C, Soussi T. Tp53 tumor suppressor gene: a model for investigating human mutagenesis. Gene Chromosome Cancer. 1992;4:1–15.
33. Hollstein M, Sidransky D, Vogelstein B, Harris CC. P53 mutations in human cancers. Science. 1991;253:49–53.
34. Soussi T, Legros Y, Lubin R, Ory K, Schlichtholz B. Multifactorial analysis of p53 alteration in human cancer: a review. Int J Cancer. 1994;57:1–9.
35. Storey A, Thomas M, Kalita A, Harwood C, Gardiol D, Mantovani F, et al. Role of a p53 polymorphism in the development of human papillomavirus-associated cancer. Nature. 1998;393:229–34.
36. Soulitzis N, Sourvinos G, Dokianakis DN, Spandidos DA. P53 codon 72 polymorphism and its association with bladder cancer. Cancer Lett. 2002;179:175–83.
37. Huang SP, Wu WJ, Chang WS, Wu MT, Chen YY, Chen YJ, et al. P53 codon 72 and p21 codon 31 polymorphisms in prostate cancer. Cancer Epidemiol Biomarkers Prev. 2004;13:2217–24.
38. Henner WD, Evans AJ, Hough KM, Harris EL, Lowe BA, Beer TM. Association of codon 72 polymorphism of p53 with lower prostate cancer risk. Prostate. 2001;49:263–6.
39. Wu WJ, Kakehi Y, Habuchi T, Kinoshita H, Ogawa O, Terachi T, et al. Allelic frequency of p53 gene codon 72 polymorphism in urologic cancers. Jpn J Cancer Res. 1995;86:730–6.
40. de Roda Husman AM, Walboomers JM, van den Brule AJ, Meijer CJ, Snijders PJ. The use of general primers gp5 and gp6 elongated at their 3' ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR. J Gen Virol. 1995;76(Pt 4):1057–62.
41. de Araujo MR, De Marco L, Santos CF, Rubira-Bullen IR, Ronco G, Pennini I, et al. Gp5+/6+ SYBR green methodology for simultaneous screening and quantification of human papillomavirus. J Clin Virol. 2009;45:90–5.
42. Cubie HA, Seagar AL, McGoogan E, Whitehead J, Brass A, Arends MJ, et al. Rapid real time PCR to distinguish between high risk human papillomavirus types 16 and 18. Mol Pathol. 2001;54:24–9.
43. van den Brule AJ, Pol R, Fransen-Daalmeijer N, Schouls LM, Meijer CJ, Snijders PJ. Gp5+/6+ PCR followed by reverse line blot analysis enables rapid and high-throughput identification of human papillomavirus genotypes. J Clin Microbiol. 2002;40:779–87.
44. Schmitt M, Dondog B, Waterboer T, Pawlita M. Homogeneous amplification of genital human alpha papillomaviruses by PCR using novel broad-spectrum gp5+ and gp6+ primers. J Clin Microbiol. 2008;46:1050–9.
45. Roberts CC, Swoyer R, Bryan JT, Taddeo FJ. Comparison of real-time multiplex human papillomavirus (HPV) PCR assays with the linear array HPV genotyping pcr assay and influence of DNA extraction method on HPV detection. J Clin Microbiol. 2011;49:1899–906.
46. ACS. Estimated 2013 cancer deaths by site, sex, & age group. Am Cancer Soc, Surveill Res. 2013
47. Parkin DM. The global health burden of infection-associated cancers in the year 2002. Int J Cancer. 2006;118:3030–44.
48. ACS. What are the key statistics about prostate cancer? Am Cancer Soc. 2013.
49. Scheffner M, Werness BA, Huibregtse JM, Levine AJ, Howley PM. The e6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. Cell. 1990;63:1129–36.
50. Scheffner M, Huibregtse JM, Vierstra RD, Howley PM. The HPV-16 e6 and e6-ap complex functions as a ubiquitin-protein ligase in the ubiquitination of p53. Cell. 1993;75:495–505.
51. Klug SJ, Ressing M, Koenig J, Abba MC, Agorastos T, Brenna SM, et al. Tp53 codon 72 polymorphism and cervical cancer: a pooled analysis of individual data from 49 studies. Lancet Oncol. 2009;10: 772–84.
52. Sonoyama T, Sakai A, Mita Y, Yasuda Y, Kawamoto H, Yagi T, et al. Tp53 codon 72 polymorphism is associated with pancreatic cancer risk in males, smokers and drinkers. Mol Med Rep. 2011;4:489–95.
53. Alawadi S, Ghabreau L, Alsaleh M, Abdulaziz Z, Rafeek M, Akil N, et al. P53 gene polymorphisms and breast cancer risk in Arab women. Med Oncol. 2011;28:709–15.
54. Wang JJ, Zheng Y, Sun L, Wang L, Yu PB, Dong JH, et al. Tp53 codon 72 polymorphism and colorectal cancer susceptibility: a meta-analysis. Mol Biol Rep. 2011;38:4847–53.
55. Jiang DK, Yao L, Ren WH, Wang WZ, Peng B, Yu L. Tp53 arg72pro polymorphism and endometrial cancer risk: a meta-analysis. Med Oncol. 2011;28:1129–35.
56. Huang CY, Su CT, Chu JS, Huang SP, Pu YS, Yang HY, et al. The polymorphisms of p53 codon 72 and mdm2 snp309 and renal cell carcinoma risk in a low arsenic exposure area. Toxicol Appl Pharmacol. 2011;257:349–55.
57. Yu H, Huang YJ, Liu Z, Wang LE, Li G, Sturgis EM, et al. Effects of mdm2 promoter polymorphisms and p53 codon 72 polymorphism on risk and age at onset of squamous cell carcinoma of the head and neck. Mol Carcinog. 2011;50:697–706.
58. Piao JM, Kim HN, Song HR, Kweon SS, Choi JS, Yun WJ, et al. P53 codon 72 polymorphism and the risk of lung cancer in a Korean population. Lung Cancer. 2011;73:264–7.

59. Dokianakis DN, Papaefthimiou M, Tsiveleka A, Spandidos DA. High prevalence of HPV18 in correlation with ras gene mutations and clinicopathological parameters in cervical cancer studied from stained cytological smears. Oncol Rep. 1999;6:1327–31.
60. Brooks LA, Tidy JA, Gusterson B, Hiller L, O'Nions J, Gasco M, et al. Preferential retention of codon 72 arginine p53 in squamous cell carcinomas of the vulva occurs in cancers positive and negative for human papillomavirus. Cancer Res. 2000;60: 6875–7.
61. Rosenthal AN, Ryan A, Al-Jehani RM, Storey A, Harwood CA, Jacobs IJ. P53 codon 72 polymorphism and risk of cervical cancer in UK. Lancet. 1998;352:871–2.