Short Communication

TP53 Polymorphism, HPV Infection, and Risk of Cervical Cancer

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Abstract

The role of a polymorphism at position 72 of the tumor suppressor gene *TP53* in the development of cervical cancer is not well established. The arginine variant of the p53 protein could be more susceptible to degradation by human papillomavirus (HPV) E6 protein than the protein containing proline. Recent studies show controversial results. We investigated a possible association between TP53 polymorphism and cervical cancer in a Peruvian population with high prevalence of HPV infection. HPV status and TP53 polymorphism were determined for 119 cases of invasive cervical cancer and 127 control women from Peru. HPV infection was detected by PCR of cervical cells or tumor biopsies. For determination of TP53 polymorphism, exon 4 of the *TP53* gene was amplified by PCR, and DNA was subsequently subjected to restriction enzyme digest. Associations between TP53 polymorphism, HPV infection, and cervical cancer were assessed using logistic regression. Women homozygotes for arginine had a 2.2-fold increased risk (95% confidence interval: 0.6–7.6) for cervical cancer. The odds ratio for women heterozygotes for Arg/Pro was 3.5 (95% confidence interval: 0.9–14). Similarly increased risks were found when restricting analysis to HPV-positive women only. The distribution of TP53 genotypes in this Peruvian population was comparable with that found in Caucasians. Our results cannot rule out an association between the TP53 polymorphism at codon 72, HPV infection, and the etiology of cervical cancer.

Introduction

There is substantial epidemiological and experimental evidence indicating that infection with certain types of HPV[2] is the main cause of cervical cancer (1, 2). In addition, our recent observations suggest that HPV is a necessary cause of this cancer (3). However, the fact that only a fraction of women infected with these viruses progresses to cancer indicates that HPV is not a sufficient cause and points to the role of cofactors. Genetic susceptibility is likely to be an important cofactor.

A polymorphism at codon 72 of the human tumor-suppressor gene *TP53* (OMIM 1911170), resulting in translation to either Arg or Pro, has been investigated as a genetic marker for the risk to develop cervical cancer. The p53 protein is one of the most important cellular proteins in the defense against tumor growth (4). After HPV infection, viral E6 protein binds to cellular p53 protein (5). Biological and biochemical differences between the p53 protein containing Arg or Pro at amino acid 72, which have recently been reported, allow consideration of a different interaction pattern between either p53 protein variant and the E6 protein of HPV (6, 7).

Storey *et al.* (8) suggested that women homozygous for the Arg allele were about seven times more susceptible to cervical cancer than Arg/Pro heterozygous women. They compared 41 normal tissues with 30 HPV-positive cervical tumor biopsies. However, most of 28 studies[3] reported failed subsequently to confirm the findings of Storey. Zehbe *et al.* (9) described a higher proportion of HPV 16-positive cervical cancer cases among women homozygotes for Arg in an Italian and a Swedish group. However, in this study, the number of invasive cervical cancer cases did not exceed 30 in either group. Two Greek authors also found increased risks for Arg/Arg homozygous women in their studies (10, 11).

The associations between cervical cancer and TP53 polymorphism were assessed in a case-control study in Peru.

Materials and Methods

Study Population. A hospital-based case-control study investigating HPV, other risk factors, and cervical cancer was conducted in Peru.[4] Study subjects were women aged 21–84 years, residents of Lima, and recruited from two hospitals in the Lima metropolitan area between April 1996 and July 1997. Cases recruited were defined as women with newly diagnosed, histologically confirmed invasive cervical cancer, who were in reasonable physical and mental condition. Controls were recruited from women without cervical cancer, history of conization, hysterectomy, or diseases associated with known risk factors for cervical cancer. Controls were matched to cases within 10-year age groups. A standardized questionnaire was administered by trained female interviewers. Cases (198) of invasive cervical cancer and 196 control women provided exfoliated cervical cells (controls) and tumor biopsies (cases). WBCs were obtained from 119 cases and 127 controls which had adequate quality for molecular analysis of TP53 polymor-

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[2] The abbreviations used are: HPV, human papillomavirus; Arg, arginine; Pro, proline; OR, odds ratio; CI, confidence interval.

[3] Because of the reference limitation, not all publications were cited here. A complete list can be obtained from the corresponding author.

[4] C. Santos *et al.*, in press, British Journal of Cancer.

phism. No differences between subjects of the entire case-control population and the study population presented here were found regarding age, ethnic group, and education. There were slightly less HPV-positive controls in the study population (13.4%), compared with the entire case-control population (17.7%).

Laboratory Methods

Analysis of TP53 Polymorphism. DNA extracted from WBCs using QIAamp spin columns (Qiagen) was amplified using a two-step PCR reaction. Codon 72 polymorphism was analyzed by digestion with restriction enzyme *Acc*II (Amersham), which cleaves a CG/CG sequence in the Arg variant of codon 72. The status was confirmed by direct sequencing, as well as by allele-specific PCR (8) in 24 randomly selected subjects.

Detection of HPV Infection. HPV infection was detected by GP5+/6+ primer-mediated PCR of cervical cells for the controls and tumor biopsies for the cases (12). Subsequent HPV typing was performed by Southern blot hybridization with different specific oligonucleotide probes able to determine 33 genital HPV types (13).

Statistical Analysis. Unconditional logistic regression was applied using Intercooled STATA 6 (Statistical Software Package version 6.0) to calculate ORs for the association between cervical cancer and TP53 polymorphism. The Pro/Pro genotype was used as the reference category. ORs were calculated for genotypes Arg/Arg and Arg/Pro independently. Analysis of association using all women was adjusted for age, HPV infection, and ethnic group. Analysis limited to HPV-positive women was adjusted for age and ethnic group. Additional variables available in the questionnaire were not considered in these analyses to limit instability of the models because of small numbers in some strata.

The *a priori* hypothesis was that women with an Arg/Arg genotype have a 7-fold higher risk to develop cervical cancer (8). Considering a comparison between the Arg/Arg and Pro/Pro genotypes, a sample size of 154 subjects (77 cases and 77 controls) was required to detect an OR of 7 with a power of 90%. This was achieved in our analysis including all women.

Results

Selected characteristics of the study population are summarized in Table 1. The majority of the women were of Mestizo origin (89%), a mix of native people of Peru and Spanish settlers, with no case-control difference. HPV positive were 94.1% of the cases and 13.4% of the controls. The most frequent HPV types found were 16, 18, 31, 35, 45, and 52, all considered high-risk HPV types (Table 1). The age-adjusted OR for HPV-positive women was 113 (95% CI: 43–303), independent from TP53 genotype.

The prevalence of TP53 genotypes in this population is 51.2% Arg/Arg, 36.6% Arg/Pro, and 12.2% Pro/Pro. This distribution is similar to the one found in Caucasian women (14–16) but differs from women in Costa Rica (15) or Japan (17). The genotype frequencies observed in cases and controls are in Hardy-Weinberg equilibrium.

Investigating the association between TP53 polymorphism and invasive cervical cancer, no difference was found between crude ORs (data not shown) and age-adjusted ORs (Table 2). After adjusting for HPV infection, the risk of Arg/Arg homozygotes for cervical cancer was 2.2-fold increased compared with Pro/Pro homozygotes (95% CI: 0.6–7.6; Table 2). However, this increase was statistically not significant. Arg/Pro heterozygotes had a 3.5-fold increased risk compared with the Pro/Pro reference category (Table 2). This increase is of borderline statistical significance (95% CI: 0.9–14), but the CI is wide. Adjusting additionally for ethnic group did not influence ORs.

Table 1 Characteristics of invasive cervical cancer cases and controls

	Cases *n* (%)	Controls *n* (%)	P^a
All women	119 (100)	127 (100)	
Age (yrs)			0.478
<40	29 (24.4)	23 (18.1)	
40–49	37 (31.1)	44 (34.6)	
50–59	25 (21.0)	34 (26.8)	
≥60	28 (23.5)	26 (20.5)	
Ethnic groups			0.407
Mestizo	109 (91.6)	111 (87.4)	
White	6 (5.0)	12 (9.4)	
Black	4 (3.4)	3 (2.4)	
Asian		1 (0.8)	
HPV positive			0.000
Yes	112 (94.1)	17 (13.4)	
No	6 (5.1)	100 (78.7)	
Missing[b]	1 (0.8)	10 (7.9)	
HPV type distribution[c]			
HPV 16	60 (50.4)	2 (1.6)	
HPV 18	18 (15.1)	4 (3.2)	
HPV 31	10 (8.4)	2 (1.6)	
HPV 35	5 (4.2)	1 (0.8)	
HPV 45	6 (5.0)		
HPV 52	9 (7.6)	2 (1.6)	

[a] Two-sided χ^2 test.
[b] Available materials did not contain DNA of high enough quality for amplification.
[c] Both single and multiple infection were counted; only the most frequent HPV types are shown here. Note that all are high-risk oncogenic HPV types.

The same tendencies, although not statistically significant, were observed by restricting analysis to only HPV-positive women. ORs, crude (data not shown), as well as after adjusting for age, were 2.2 (95% CI: 0.6–8.6) for Arg/Arg and 2.6 (95% CI: 0.6–11) for Arg/Pro (Table 2). Additionally adjusting for ethnic group did not change ORs.

Discussion

Our results do not support the initial findings of Storey *et al.* (8), suggesting that individuals with Arg/Arg genotype had a 7-fold increased risk to develop HPV-associated cervical cancer. However, the data reported here suggest an increased risk associated with the presence of at least one Arg allele. There is an increased risk, although not statistically significant, in homozygotes for Arg/Arg and additionally in heterozygotes for Arg/Pro compared with homozygotes for Pro/Pro. Similar results were obtained when restricting the analysis to HPV-positive cases and controls. The majority of HPV-positive cases and controls (96.4% of HPV-positive cases and 64.7% of HPV-positive controls) was infected with HPV types considered high risk (Table 1).

At least two of the published studies, which did not report an increased risk for women carrying two Arg alleles, actually found associations with cervical cancer risk when analysis was limited to subgroups of HPV 16- or 18-positive women (14) or only HPV 18-positive women (15). Helland *et al.* (14) reported a slightly increased OR (not significant) for Arg/Arg homozygote HPV 16- or 18-positive cervical carcinomas. Hildesheim *et al.* (15) reported a weak, nonsignificant association between

Table 2 TP53 frequency and association with invasive cervical cancer in Peruvian women

	Genotype	Cases *n* (%)	Controls *n* (%)	OR [95% CI][a]	OR [95% CI][b]
All women		119 (100)	127 (100)		
	Pro/Pro	14 (11.8)	16 (12.6)	1.0 (ref. cat.)	1.0 (ref. cat.)
	Arg/Pro	45 (37.8)	45 (35.4)	1.1 [0.5–2.6]	3.5 [0.9–14]
	Arg/Arg	60 (50.4)	66 (52.0)	1.0 [0.5–2.3]	2.2 [0.6–7.6]
HPV-positive women only		112 (100)	17 (100)		
	Pro/Pro	13 (11.6)	4 (23.5)	1.0 (ref. cat.)	
	Arg/Pro	41 (36.6)	5 (29.4)	2.6 [0.6–11]	
	Arg/Arg	58 (51.8)	8 (47.1)	2.2 [0.6–8.6]	

[a] OR adjusted for age.
[b] OR adjusted for age and HPV.

p53 status and cervical neoplasia if they restrict their analysis to HPV 18-positive women. In a recent analysis of the E6 variants of HPV 16 in relation to the TP53 codon 72 polymorphism, van Duin *et al.* (18) reported a small, statistically not significant increase in risk of cervical carcinoma among HPV 16-positive, Arg/Arg homozygous women. They found an overrepresentation of the HPV 16 350T variant compared with the 350G variant in Arg/Arg homozygous women with cervical cancer. In our study, ORs for women positive for HPV 16 and/or HPV 18 only was not computed separately because of very small numbers of controls positive for HPV 16 and/or 18.

Interestingly, we found an increase in risk associated with the Arg/Pro genotype. Such an association has only been substantiated in one other study (19), and, at present, the biological basis for increased susceptibility in Arg/Pro heterozygote individuals remains obscure.

There are several major problems of comparability between the different cited studies. Briefly, the ethnicity of the subjects under investigation in the different publications varies greatly. Inclusion criteria of cases and controls are often complying to epidemiological standards, but sometimes, they are mere case series with unclear selection of controls (8, 20), such as one study including male subjects as controls (21). The greatest problem for drawing general conclusions out of these studies is the diversity of information on HPV status of the subjects. Some of the publications did not ascertain HPV status (22–25). Only in a few publications, statistical analysis restricted for HPV-positive women was computed (14–16, 26). The DNA source to assess TP53 polymorphism and, if performed, HPV status, differs greatly between WBCs, cervical cells, tumor biopsies, paraffin-embedded tissue, or remains unreported. The use of tumor cells is a source of error, as their DNA may often have lost one allele at the TP53 locus. Another potential problem is the variety of different laboratory techniques used for the detection of TP53 polymorphism. Moreover, it has been shown that even using the same technique to detect TP53 polymorphism in different laboratories leads to substantial variation in results (26). In the statistical analysis, different authors used different genotypes or genotype combinations as reference categories when calculating ORs to estimate risk. From the biological point of view, it seems inappropriate to use the Arg/Pro heterozygote phenotype as the reference category or as part of it when computing risks for the homozygote genotypes (8).

In future studies, it will be reasonable to specifically address the role of codon 72 polymorphism in individuals positive for high-risk HPV types or HPV 16 and/or 18 only and relate the results to the variants of these HPV types. The biological rationale behind the hypothesis is that the variant of p53 protein containing Arg at codon 72 is highly sensitive to degradation by E6 protein of HPV 16 or 18. Recent evidence indicates that the two p53 protein variants show a number of functional differences in their abilities to bind components of the transcriptional machinery, activate transcription, induce apoptosis, and repress the transformation of primary cells (6).

The main limitation of our study is small numbers in the individual categories after grouping and stratification. Chance association cannot be ruled out. However, this study is an important contribution to the ongoing discussion on TP53 polymorphism and cervical cancer. Future investigations with large numbers of high-risk HPV-positive subjects recruited in accordance to epidemiological criteria are needed to determine the possible role of this polymorphism.

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