

# TP53 R72P Polymorphism and Susceptibility to Human Papillomavirus Infection Among Women With Human Immunodeficiency Virus in Morocco: A Case-control Study

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**Background:** Human papillomavirus (HPV) is the most common sexually transmitted agent worldwide. HPV is the main causative agent for cervical cancer. The HPV oncoprotein E6 binds to the tumor suppressor gene product p53, promoting its degradation; the Arg allele of TP53 R72P polymorphism binds more ardently with HPV E6 than the Pro variant. Here, we investigated whether TP53 R72P gene variant, rs104252, was associated with susceptibility to HPV infection in women with human immunodeficiency virus (HIV).

**Methods:** We analyzed 200 HPV-positive and 68 uninfected women with HIV. Genomic DNA was isolated from cervical swab. The TP53 R72P polymorphism was genotyped by PCR-RFLP. Unconditional logistic regression was used to assess the association between polymorphism and the clinical, lifestyle, and behavioral data.

**Results:** The genotype and allele frequencies of rs104252 variant did not differ between women without or with HPV infection ( $P > 0.05$ ). Moreover, the p53 polymorphism was not associated with cervical cytology. In contrast, when we analyzed according to behavior factors, the P72P genotype was more frequent among HPV-positive smoker women. However, no significant relationship was found between alcohol, contraceptive use, and number of partners with TP53 R72P genotype distributions among HPV-positive cases ( $P > 0.05$ ).

**Conclusions:** The R72 variant of p53 R72P is not associated with HPV infection and progression of lesions. There was no association between this variant and behavior factors in HPV-positive cases. The P72P genotype may be more frequent among HPV-positive smoker women.

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**Key Words:** P53 codon 72, Human papillomavirus, Polymorphism, Susceptibility, Human immunodeficiency virus

## INTRODUCTION

Human papillomavirus (HPV) infections are very common sexually transmitted infections that comprise more than 200 types.<sup>1,2</sup> Although the majority of HPV infections resolve or become latent and undetectable, the infection persists in a subset of women.<sup>3,4</sup> Experimental and clinical evidences demonstrate that the immunological and genetic backgrounds of the host play

an important role in the outcome of HPV infection.

One host gene that interacts with HPV and promotes cancer development is p53 tumor suppressor. Translation of the high-risk HPV E6-E6AP complex leads to the proteosomal degradation of p53 through the ubiquitin-proteasome pathway and then allows viral replication by inhibiting p53-mediated antiviral responses.<sup>5,6</sup> The TP53 gene is located at human chromosome 17 and encodes a 53-kDa nuclear phosphoprotein,

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which plays crucial roles in cell cycle regulation, maintenance of genomic integrity, apoptosis, and challenge of environmental insult but also in the innate host immune control of viral infections by orchestrating diverse signaling pathways from many different cellular receptors and sensors.<sup>7</sup> A common polymorphism in codon 72 of TP53 is the exchange from CCC, which encodes proline (P72), to CGC, which encodes for arginine (R72). This polymorphism is located within the proline-rich region of p53.<sup>8</sup> These allelic variants differ in their functional ability to activate transcription, induction of apoptosis, DNA methylation, and transformation of primary cells.<sup>9,11</sup> Furthermore, previous studies have shown that the R72 variant binds to the high-risk HPV E6 protein with greater efficiency than the P72 variant.<sup>12,14</sup> In addition, *in vitro* data support a functional relationship between p53 and human immunodeficiency virus (HIV) proteins with evidence for implications for p53-mediated apoptosis.<sup>15,16</sup>

To our knowledge, few studies to date have evaluated TP53 polymorphism and susceptibility to HPV infection in HIV infected women. Thus, we investigated the association of TP53 R72P SNP with viral persistence of HPV infection and for the development of neoplastic lesions in Moroccan women living with HIV.

## MATERIALS AND METHODS

### 1. Study population

A case-control study was carried out. A written informed consent was obtained from all individuals. Each participant completed a structured questionnaire on clinical and demographic data. The protocol was approved by the Ethics Committee of the Faculty of Medicine of Casablanca and the study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki. Cervical swab specimens were obtained from 268 HIV-infected women from different geographic regions of Morocco. Patients were followed at the Infectious Disease Center, University Hospital Center, Ibn Rochd in Casablanca.

### 2. Clinical laboratory data and cytologic analysis

CD4<sup>+</sup> T-cell counts were enumerated by flow cytometry on a three-color FACSCallibur flow cytometer (Becton Dickinson Immunocytometer System, San Jose, CA, USA). For HIV RNA viral load, automated extraction, amplification, and quantification were performed with the Cobas Ampliprep/Cobas TaqMan 48 analyzer system ver. 2.0 (Roche Diagnostics, Ltd., Rotkreuz, Switzerland) following the Roche manufacturer's standard

guidelines.

The Papanicolaou smear on the slide in a monolayer was performed for cytological study. Smear abnormalities were classified using Bethesda system. The remaining of samples were stored in transport cell solutions, general cytology preservative medium (Cell Solutions, Greensboro, NC, USA) at  $-20^{\circ}\text{C}$  until use.

### 3. DNA isolation, human papillomavirus testing, and genotyping of p53 codon 72

DNA was isolated from the cervical swab specimens using QIAamp blood DNA Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The samples were screened for the presence of HPV using nested PCR as described previously.<sup>17-19</sup> HPV typing was performed by direct sequencing as described previously.<sup>17,19</sup> The genotyping of p53 codon 72 was performed by PCR-RFLP as reported previously.<sup>20</sup>

### 4. Statistical analysis

Departure from Hardy-Weinberg equilibrium was determined by comparing the observed genotype frequencies with expected genotype frequencies in all groups calculated, using observed allele frequencies by freedom of chi-square goodness-of-fit test with 1 degree. Comparisons of continuous variables were assessed with the Mann-Whitney U-test, whilst those between categorical variables were evaluated using the chi-square and Fisher's tests. Comparisons of continuous variables between groups were assessed with the Mann-Whitney U-test. The association between case-control status measured by the OR and its corresponding 95% CI was estimated using an unconditional logistic regression model. A *P*-value  $< 0.05$  was considered statistically significant. All tests were two-sided. Statistical analyses were performed using GraphPad PRISM ver. 6.0e (GraphPad Software, San Diego, CA, USA) and IBM SPSS software package ver. 20.0 (IBM Co., Armonk, NY, USA).

## RESULTS

According to HPV status, we analyzed 200 HPV-positive cases and 68 uninfected women with HIV as controls. Selected characteristics of participants are presented in Table 1. Smoking status was statistically significant between cases and controls. In the HPV-positive group, 55.98% (112 cases) were R72R, 31.50% (63 cases) were R72P, and 12.50% (25 cases) were P72P. In the HPV-negative group, 63.24% (43 cases) were R72R, 27.94% (19 cases) were R72P, and 8.82% (6 cases) were P72P (Table 1).

**Table 1.** Baseline socio-demographic, reproductive lifestyle, and genetic characteristics of study population

Characteristic	HPV-negative subset (n = 68)	HPV-positive subset (n = 200)	Pvalue
Age (yr)	41.5 (23-67)	38.0 (20-77)	0.128
Smoker	13 (19.35)	61 (30.43)	< 0.0001
Alcohol intake	16 (24.19)	57 (28.26)	0.426
Oral contraceptive use	45 (66.13)	135 (67.00)	0.841
Age at first intercourse (yr)	19 (12-38)	19 (11-40)	0.764
Age of first pregnancy (yr)	24 (13-40)	22 (13-46)	0.475
Multiple sex partners	33 (48.39)	112 (55.98)	0.285
HAART	68 (100)	196 (97.83)	0.575
CD4+ T count (cell/mm <sup>3</sup> )	477 (29-1,517)	535 (5-1,896)	0.098
Viral load (log <sub>10</sub> copies/mL)	1.60 (1.30-6.81)	1.60 (1.30-5.88)	0.632
HIV stage			
A	21 (30.65)	73 (36.41)	0.557
B	20 (29.03)	37 (18.48)	0.057
C	27 (40.32)	90 (45.11)	0.447
TP53 R72P polymorphism			
R72R	43 (63.24)	112 (56.00)	0.322
R72P	19 (27.94)	63 (31.50)	0.649
P72P	6 (8.82)	25 (12.50)	
R	0.772 ± 0.039	0.718 ± 0.025	
P	0.228 ± 0.039	0.282 ± 0.025	0.215

Values are presented as median (range), number (%), or mean ± SD. HPV, human papillomavirus; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus.

**Table 2.** Distribution of TP53 R72P polymorphism genotypes among HPV-positive cases according PAP smear results

TP53 R72P polymorphism	Abnormal lesion (n = 117)	Normal lesion (n = 63)	OR (95% CI)	P-value
R72R	64 (54.70)	38 (60.32)	0.47 (0.16-1.36)	0.157
R72P	35 (29.91)	20 (31.75)	0.49 (0.16-1.51)	0.207
P72P	18 (15.38)	5 (7.94)	1 (reference)	
R	0.697 ± 0.034	0.762 ± 0.040	0.72 (0.44-1.18)	0.188
P	0.303 ± 0.034	0.238 ± 0.040	1 (reference)	

Values are presented as number (%) or mean ± SD. HPV, human papillomavirus; PAP, papanicolaou.

Hardy-Weinberg analysis showed equilibrium in both groups ( $P > 0.05$ ). The R72R genotype was more common in HPV-negative samples than in HPV-positive cases (63.24 vs. 56.00%) but the difference was not statistically significant ( $P = 0.322$ ). Moreover, the allelic distribution did not show a significant difference between groups.

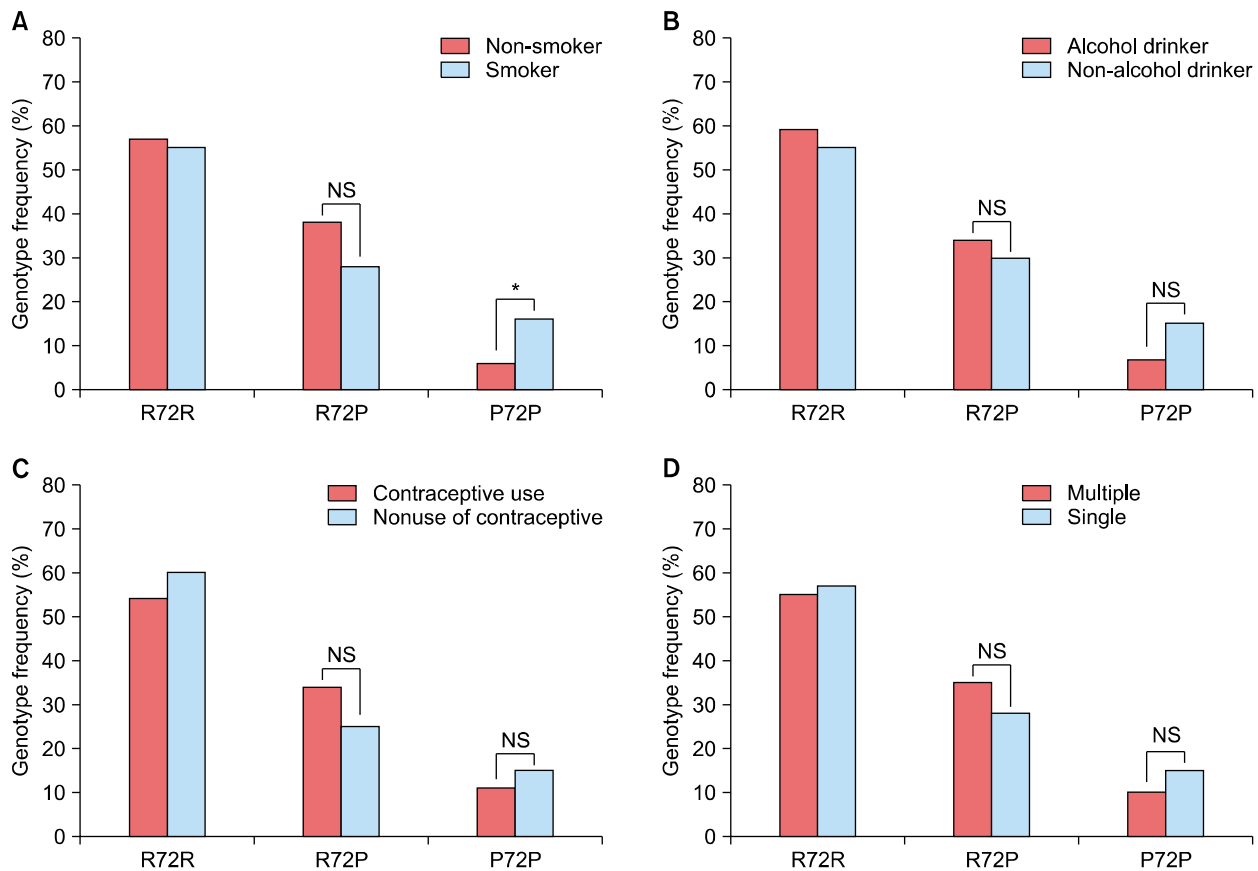
Stratification of HPV-positive cases according to cervical cytology findings was performed (Table 2). In the abnormal specimens (atypical squamous cells of undetermined significance, low-grade squamous intraepithelial lesion, and high-grade intraepithelial lesion), the frequency for the R72R genotype was 54.70%, the proportion of R72P genotype was 29.91%, and 15.38% were homozygous for the P allele (Table 2). Non statistically significant differences were found for the frequencies of the different p53 genotypes and allele in the different cytological

groups ( $P > 0.05$ ). Finally, between abnormal cases and normal controls, there was no an increase of risk to more cervical lesions in genotypes or allele distribution (Table 2).

Next, we analyzed R72P polymorphism according to behavior factors in HPV-positive women (Fig. 1). Overall, the P72P genotype was more frequent among HPV-positive smoker women ( $P = 0.027$ ) (Fig. 1A). Whereas, the genotype frequencies of TP53 R72P polymorphism was not associated with other risk factors (alcohol, contraceptive use and number of partners) among HPV-positive cases ( $P > 0.05$ ) (Fig. 1B-1D).

## DISCUSSION

Previous evidence has suggested that p53 protein containing arginine at codon 72 is more susceptible to elimination by the E6



**Figure 1.** Genotypes of R72P polymorphism according to behavior factors in human papillomavirus-positive women. (A) Genotypes frequencies of R72P and smoking habits. (B) Genotypes frequencies of R72P and alcohol intake. (C) Genotypes frequencies of R72P and contraceptive use. (D) Genotypes frequencies of R72P and number of partners. \* $P < 0.05$ .

protein of oncogenic HPV. This mechanism could possibly cause increased susceptibility to infection with HPV and promote cancer development in infected individuals.<sup>12</sup> Moreover, previous in vitro study showed that the HIV-1 Tat protein seems to promote the cellular proliferation and positively regulate the E6 and E7 HPV genes, responsible for the malignant cellular transformation.<sup>21</sup> In the present study, we tested the hypothesis that functional polymorphisms in gene encoding p53 might influence the susceptibility to HPV infection and/or the susceptibility to progression of HPV-related cervical lesions in women living with HIV-1. Our study showed no association between proline homozygosity and HPV infection, and thus do not support the hypothesis that the p53 polymorphism is associated with an increased risk of HPV persistence. This data corroborates with previous study.<sup>22</sup> In recent Brazilian studies, authors found no statistically significant relation between observed genotypes, frequency of the Arg and Pro alleles and HPV presence in HIV-infected women.<sup>23,24</sup> Furthermore, in uninfected-HIV women, several studies reported that polymorphism at codon 72 of TP53

gene is not associated with an increased susceptibility to HPV infection.<sup>25-27</sup>

Our result confirms that the TP53 polymorphism has no effect on the development of preinvasive lesions. This study adds further evidence to other surveys proving that there is no association between TP53 R72P polymorphism and cervical precancerous lesions.<sup>25-28</sup> Because we did not study women with invasive cervical cancer, we cannot exclude that the TP53 R72P SNP may act at later stages of carcinogenesis.

Interestingly, when we analyzed R72P polymorphism according to behavior factors in HPV-positive women. Only tobacco intake was found to bear a positive relation with P72P genotype ( $P = 0.026$ ). Previous reports revealed a significant synergistic effect between the p53 polymorphism and smoking on cancer risk.<sup>29-31</sup>

In conclusion, this study suggests that polymorphism at codon 72 of TP53 gene is not associated with an increased susceptibility of HPV infection and/or to cervical disease in the Moroccan women living with HIV/acquired immunodeficiency syndrome

(AIDS). This finding was not modified by concurrent HIV. The small sample size is a major limitation in this study. There were no research articles investigating the association between HPV infection and p53 codon 72 polymorphism among patients living with HIV-1. Thus, additional studies are needed with larger sample size to reevaluate the impact of TP53 R72P polymorphism on HPV persistence and HPV-associated cervix cancer susceptibility in women living with HIV/AIDS.

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## CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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