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## Research Article

# Molecular Analysis of P53 Codon 72 Polymorphism and Risk of Cervical Carcinoma among Women in Southwest of the Republic of Congo: A Case-Control Study -

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## ABSTRACT

A common polymorphism of *p53* gene at codon 72 in exon 4 has been associated with increased risk in wide variety of human cancers. The arginine homozygous genotype has been reported as risk factor to HPV-related cervical carcinoma, although many studies have failed to confirm this hypothesis. This study aimed to analyze the role of *p53* codon 72 polymorphism for cervical carcinoma risk in Congolese women from southwest of Congo. Samples from 106 women, 56 cervical carcinomas confirmed histologically and 50 healthy controls were analyzed by allele-specific PCR assay. HPV-DNA were detected by nested-PCR using MY09/MY11 and GP5+/GP6+ universal primers, followed by typing with type-specific primers for HPV 16 and 18. HPV-DNA was detected in 96.4% (54/56) of case group and 32% (16/50) of control. HPV16 and HPV18 were detected in 62.9% and 7.4% in the case group, and in 43.7% and 6.2% in control. Genotypes frequencies of Arg/Arg, Arg/Pro and Pro/Pro were 69.6, 21.4 and 9.0% in the cervical carcinomas and 52.0, 40.0 and 8.0% in the control. Allele frequencies of Arg and Pro were 0.80 and 0.20 in case group and 0.72 and 0.28 in the control respectively. We observed no significant association in the distribution of *p53* codon 72 genotypes polymorphism between cases versus control group ( $p > 0.05$ ). Our results showed that the codon 72 polymorphism *p53* Arg would not be associated with an increased risk of developing cervical carcinoma among women in the Southwestern of Congo.

**Keywords:** *p53* Codon 72 Polymorphism; Cervical carcinoma; HPV; Southwest Congo

## INTRODUCTION

Cervical Cancer (CC) is the third most frequent malignancy in women worldwide. More than 85% of cases occur in developing countries [1]. Age-standardized incidence rate in the Republic of Congo is estimated at 25.2 [2]. Several epidemiological and molecular data showed clearly that the Human Papilloma Virus (HPV), particularly high-risk types are the causative agents of CC [3-5]. However, most cervical HPV-infections disappear spontaneously and only a small fraction of infected women develop cervical carcinoma [6]. This fact shows that HPV infection is necessary although not sufficient to cancer development. Other cofactors are essential, such as sexual behavior, hormonal factors, diet or genetic predisposition, of which the polymorphism of *p53* gene [7]. On the other hand, it is known that the E6-HPV gene binds to the *p53* protein and induces its degradation by the ubiquitin-dependent pathway [3,8-10]. This process is necessary for the immortalization of cervical epithelial cells and the progression of cancer. Indeed, the *p53* protein is a transcription factor that regulates important cellular functions. The *p53* participates in cell cycle regulation, apoptosis or DNA repair. Several studies have shown that the *p53* is mutated or degraded in more than 50% of all human cancers [11-13]. These genetic alterations include allelic loss, mutations and epigenetic changes [11,14]. Several polymorphisms have been described in this gene; the most commonly studied is a Single Nucleotide Polymorphism (SNP) at codon 72 in exon 4. This SNP encodes for two forms structurally different of the *p53* protein [12,15]. The SNP consists in the substitution of an amino acid Proline (CCC, *p53*Pro) to an amino acid arginine (CGC, *p53*Arg) in the proline-rich region [16]. The consequence of this amino acid change is the difference in the susceptibility to malignant transformation, induction of apoptosis, and transcriptional activity [17,18]. Since the study of Storey et al. (1998), the involvement of this polymorphism in the cervical cancer development was widely studied. In this study, Storey showed that the arginine form of *p53* protein was more vulnerable than proline form to binding and degradation by the E6 HPV gene. Similarly, women who are homozygous Arg/Arg are seven times more likely to develop cervical cancer than those who are homozygous Pro/Pro [19]. However, the results of different studies on the subject remain controversial [4]. To our knowledge, the impact of the *p53* codon 72 polymorphism in cervical carcinoma development among Congolese women has not been reported yet. In the present study, we investigated the eventual association between *p53* Codon 72 Arg/Pro SNP and Cervical Carcinoma (CC) in Congolese women.

## MATERIALS AND METHODS

### Study population and design

**One hundred and six patients were included in this study:** For case group, we used DNA from tissues derived from 56 women

aged 31-72 years (average age:  $48.51 \pm 9.03$  years, median age: 47.5 years) who had a cervical carcinoma (all squamous cell carcinoma) histologically confirmed. For control group, 50 cervical scrapes from women aged 19-63 years (average age: 46.40 - 9.86 years, median age: 46.5 years), who had a normal cervical cytology were also used. The control group was selected among women who participated in the routine gynecological examination at the General Hospital of Loandjili (Pointe-noire, Congo). Inclusion criteria were normal findings by gynecological exam without any cytological abnormality (Pap smear test) and history of precancerous or cancerous lesion. A case-control study approach was used to investigate the susceptibility of the *p53* codon 72 polymorphism to increase risk of cervical cancer development in Congolese women. Informed consent was obtained for all women and study was approved by the local ethics committee of Health Research Sciences of Congo.

**DNA extraction and detection of HPV infection:** DNA extraction was performed using phenol/chloroform/isoamyl alcohol (25:24:1) method according to the method routinely used in our laboratory as previously described [20]. DNA pellet was dried and resuspended in 50  $\mu$ l of Ultra-pure PCR water (Bioline,UK). The HPV-DNA were detected by nested-PCR using MY09/MY11 and GP5+/GP6+ universal primers and screened for the presence of HPV16/18 by PCR using HPV 16 and 18 type-specific primers as previously described [21].

**Allele Specific PCR Analysis of the *p53* Polymorphism:** Genotyping of *p53* gene at codon 72 was performed by PCR method using allele specific primer pairs: *p53*-a1 (5'-TCCCCCTTGCCGTCCCAA-3') and *p53*-a2 (5'-CTGGTGCAGGGGCCACG-3') for arginine (142 bp) and *p53*-p1 (5'-GCCAGAGGCTGCTCCCC-3') and *p53*-p2 (5'-CGTGCAAGTCACAGACTT-3') for proline (178 bp). The allele specific PCR amplification (AS-PCR) was done separately for each of the two polymorphic variants (proline or arginine Alleles) with some minor modifications [22]. PCR reaction mixture (25  $\mu$ l) consisted of 2  $\mu$ l of template DNA solution (100 ng of DNA), 1X Taq Reaction buffer (200 mM Tris pH 8.4, 500 mM KCl), 2 mM of MgCl<sub>2</sub>, 0.2 mM of dNTP, 20 pmol of each primers and 1U platinum Taq polymerase (Invitrogen; Carlsbad, CA). PCR was carried out in a Perkin Elmer 2400 Gene Amp<sup>®</sup> PCR thermal Cycler (Scientific Support, Inc, Hayward, CA) at 94°C for 5 min to initial denaturation, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 59°C for 50s, and extension at 72°C for 30s. The final step of elongation was performed at 72°C for 7 min. The PCR products were analyzed by electrophoresis on a 2% agarose gel (Promega).



### STATISTICAL ANALYSIS

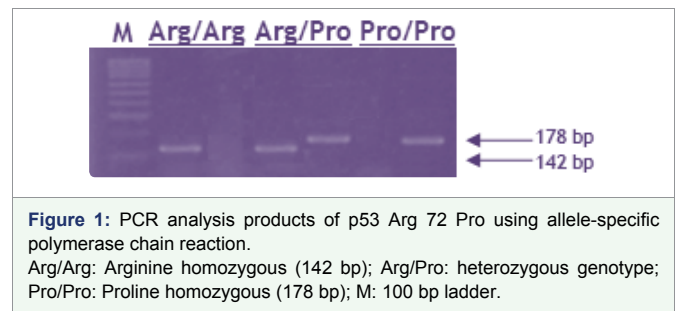
Statistical analysis was performed by Epi-info7 software (www.epivf.fr) using the Fisher's exact test or chi-square tests with and Odd ratio (OR) and its 95 % confidence interval (95 %CI) to find association and estimated risk ratio of Arg/Arg versus Arg/Pro and Pro/Pro or Pro/Pro group versus Arg/Pro and Arg/Arg between the two groups. Deviation from Hardy–Weinberg Equilibrium (HWE) in the studied groups was examined by X2 test.  $P < 0.05$  was considered to represent significant.

### RESULTS

All groups showed a good fit to the Hardy–Weinberg equilibrium ( $p > 0.05$ ). HPV infection was present in 32.0 % (16/50) of cases in control group and 96.4% (54/56) of cases in case group. HPV16/18 was detected in 70.0% and 50.0% of positives-HPV in case group and control group respectively. All results are reported in (Table 1). The (Figure 1) illustrates the PCR analysis products of p53 Arg 72 Pro using allele-specific polymerase chain reaction. The relative frequencies of alleles and genotypes distribution are summarized in (Table 1). The frequencies of alleles were: 0.80 for Arg versus 0.20 for Pro in case group; and 0.72 for Arg versus 0.28 for Pro in control group. The proportions of individual codon 72 p53 genotypes in case group versus control group were: 39/56 (69.6 %) versus 26/50 (52.0 %) for Arg/Arg; 12/56 (21.4 %) versus 20/50 (40.0 %) for Arg/Pro and 5/56 (9.0 %) versus 4/50 (8.0 %) for Pro/Pro.No significant difference was found in the distributions of p53 codon 72 genotypes among cases versus controls in increasing risk for cervical carcinoma development in our population: OR for Pro/Pro-Arg/Pro was 2.1 (95%CI 0.95–4.69;  $p = 0.06$ ) and Arg/Arg-Arg/Pro was 1.1 (95%CI 0.28–4.45;  $p = 0.86$ ).

### DISCUSSION

Genetic polymorphisms have been described as having a major role in the cancer development [23,24]. The hypothesis that certain types of p53 gene polymorphisms could represent a risk factor to develop cancer has been extensively studied. Several studies have provided evidence that the p53 polymorphism at codon 72 of exon 4 may be associated with some tumors, such as cervical cancer [25,26]. According to their study, Storey et al (1998) were the first observed that women homozygous for arginine (Arg/Arg) were approximately seven-fold more susceptible than heterozygous females to develop



cervical cancer [19]. Hence the hypothesis that, arginine genotype is an important risk factor for cervical cancer especially in the presence of HPV-infection. However, few studies have also subsequently confirmed this hypothesis [27-31], whereas others could not confirm this hypothesis [27,32-36]. In Africa, a few studies have been dedicated to assessing the impact of this polymorphism in the development of cervical cancer among some populations. However, the majority of these studies found no significant association between the polymorphism at codon 72 of p53 and susceptibility to cervical cancer [37-40]. To investigate the susceptibility of p53 codon 72 single-nucleotide polymorphism (SNP72) in the increase risk of the cervical cancer development among Congolese women, 106 genomic DNA samples from 56 cervical carcinomas cases histologically confirmed and 50 healthy controls with normal cervical cytology (Pap smear) were included for study. The genotype distribution of the p53 polymorphism fit the Hardy–Weinberg equilibrium in the cases and controls group ( $p > 0.05$ ) [41]. The results showed no significant difference in the distribution of genotype Arg/Arg versus Arg/Pro and Pro/Pro between the cases group and that of the controls ( $p = 0.06$ ). However, the relative frequency of the Arg allele was higher in cervical carcinoma group compared to the control group. Ours findings indicate that there is no association between the arginine genotype and increasing risk of cervical cancer in our studied population and seems not to confirm the first hypothesis of Storey et al. These results are consistent with several other studies in different populations of the world that could not demonstrate the initial hypothesis by studying this SNP. These conflicting results were attributed to the types of samples, the different methodologies used as well as to ethnic differences of each population [32-36]. Nevertheless, some studies has well confirmed the risk association between the p53Arg codon 72 polymorphism and development of cervical cancer as shown two meta-analysis worldwide and many other studies having investigated the issue. [4,42,43].In this study, the high tendency of Arg genotype in the patient group compared to the control group may reflect a potential risk in our population. Future studies with larger size samples would certainly help to elucidate this hypothesis. In conclusion, despite the high proportion of Arg genotype in patient group, the carriers of Arg allele in codon 72 p53 gene have not an increased risk for development of cervical carcinoma in Southwest Congolese women. However considering the small sample size of our study, a larger case-control study is necessary to confirm this trend in the general Congolese population.

### AUTHORS' CONTRIBUTIONS

ALMB conducted all handling and the overall design of the experiment. SZA participate in the critical reading of the manuscript and conducting statistical analysis. DM participated in the critical reading of the manuscript and sampling. LH and MME were responsible for the implementation of the project. All authors read and approved the final manuscript.

**Table 1:** Genotypes distribution, p53 polymorphisms relationship and cervical carcinoma risk in Southwestern Congolese women

Samples	Total n (%)	Genotypes			Alleles	
		Arg/Arg n (%)	Arg/Pro n (%)	Pro/Pro n (%)	Arg n (%)	Pro n (%)
Controlos		26 (52.0)	20 (40.0)	4 (8.0)	0.72	0.28
HPV+	16 (32.0)	8 (50.0)	6 (37.5)	2 (12.5)		
HPV-	34 (68.0)	18 (52.9)	14 (41.1)	2 (5.8)		
HPV16/18	8 (50.0)	4 (50.0)	3 (37.5)	1 (12.5)		
Cases (n=56)		39 (69.6)	12 (21.4)	5 (9.0)	0.80	0.20
HPV+	54 (96.4)	38 (70.3)	11 (20.3)	5 (9.2)		
HPV-	2 (3.6)	1 (50.0)	1 (50.0)	0 (0.0)		
HPV16/18	38 (70.3)	27 (71.0)	8 (21.0)	3 (7.8)		

Arg/Arg vs. Arg/Pro and Pro/Pro: (OR: 2.1; 95% CI 0.95 – 4.69;  $p = 0.06$ ), Pro/Pro vs. Arg/Pro and Arg/Arg: (OR: 1.1; 95% CI 0.28 – 4.45;  $p = 0.86$ ).



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