

## Research Article

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# Association of TNF- $\alpha$ -308G/A and IL-18 Polymorphisms with risk of HPV infection among sexually active women in Burkina Faso

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**Abstract:** Human papillomavirus (HPV) infection is the most common sexually transmitted infection worldwide. Persistence infection can lead to the development of cervical cancer potentially due to some genetic factors such as polymorphisms in regulatory and coding regions of cytokine genes. The purpose of this study was to determine whether there is a relationship between TNF-308 G/A or IL18 polymorphisms and high-risk HPV infection among sexually active women from Burkina Faso. Ninety-one HPV infected and two hundred and nine HPV negative women (the latter used as healthy controls) were screened. TNFA-308 G/A and IL18-607 C/A polymorphisms were detected using the TaqMan allelic discrimination. HPV 52 (21.19%), HPV 39 (11.86%) and HPV 33 (11.02%) were the most common HPV genotypes. The TNFA-308A and IL18-607 C alleles were predominant in all women in the study. None of the TNFA and IL18 alleles were associated with HPV infection. The results suggest that there is no relationship between TNF-308 G/A or IL18-607C/A polymorphisms and HPV infection among women in the study.

**Keywords:** High risk HPV; Genotype; Polymorphism; TNF $\alpha$ ; IL18.

## Introduction

Every year, many women are affected by cervical cancer, worldwide. In 2018, there were an estimated 569,847 new cases and approximately 311,365 deaths from cervical cancer which is the second most common cancer in less-developed countries [1]. This cancer usually occurs following a persistent, high-risk, HPV infection in a small proportion of women who have contracted the infection in their lifetime. Risk factors such as the age at first sexual intercourse or the number of pregnancies, as well as genetic factors, would appear to be involved in the occurrence of this disease. Some authors suggest that the host's genetic 'make-up' may facilitate the persistence of HR-HPV infection [2] or even promote the progression of infection. Cytokines such as Tumor Necrosis Factor alpha (TNF $\alpha$ ) and interleukin 18 (IL18) have been implicated in the process but the results remain controversial [3].

TNF $\alpha$  (TNFA) is a pro-inflammatory cytokine involved in systemic inflammation and acute phase reaction providing protection against infections. In humans, there is only one gene known for TNF $\alpha$  located on chromosome 6p21 within the locus of the major histocompatibility complex. TNF $\alpha$  exists in two forms, including a transmembrane form linked to the membrane which is the precursor of the soluble form [4]. Both forms are active but have different affinities for TNF receptors (TNFR-1 and TNFR-2). TNFR1 is the major receptor in the mediation of cell toxicity [5] and cell death [6]. TNFR2 is found on the surface of circulating T cells [7] but is also the primary mediator known to be involved in the self-regulation of TCD8 cell apoptosis [8]. TNF $\alpha$  presents several polymorphisms, including one in position -308 of the promoter by substitution of G in A. This single-Nucleotide Polymorphism (SNP), in addition to being involved in transcription regulation, appears to affect gene expression [9, 10].

IL18 is an important regulator of the immune response produced by dendritic cells, Langerhans

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cells, macrophages and some B lymphocyte lines [11]. In the absence of any other stimulus, IL18 increases the production of TNF  $\alpha$  by TCD4+ lymphocytes and NK cells [12]. The IL 18 gene contains several polymorphisms in the promoter region and SNPs at -137 G/C and -607 C/A positions appear to be the only ones that affect gene activity [13]. IL 18 -607C/A polymorphism was associated with protection against HPV infection while the TNFA -308A allele was associated with susceptibility to HPV infection among Brazilian women according to the study by Tavares *et al.* in 2016 [14]. Their study suggested that, it is necessary to replicate this kind of study in other populations with different ethnic backgrounds to better understand the effects of TNFA and IL18 genetic variants on HPV infection that is why we conducted this study, to investigate the distribution of TNF-308 G/A and IL18-607 C/A SNPs and their relationship to HPV infection in a cohort of women from Burkina Faso.

## Materials and methods

### Study subjects

We collected endocervical samples from women who came for gynecological consultation in hospital of two cities in the west of Burkina Faso: Bobo-Dioulasso and Orodara. The DNA extraction was made using DNA-Sorb-A kit (Sacace Biotechnologies, Como, Italy). The genotyping of high-risk HPV was performed by real-time PCR using the kit “HPV Genotypes 14 Real-TM Quant” code V67-100FRT (Sacace Biotechnologies, Como, Italy), and the Sacycler-96 Real-Time PCR (Sacace Biotechnologies, Como, Italy). [15, 16]. Following HPV genotyping, samples were separated in two groups to investigate the distribution of TNF-308 G/A and IL18-607 C/A SNPs: one which HPV infection and the other without HPV infection. Thus, this case control study involved 300 women with 91 women infected by HPV and 209 women without HPV.

### Genotyping

The polymorphic regions of TNF $\alpha$ -308 G/A (rs 1800629) and IL 18-607 C/A (rs 1946518) were amplified by PCR, with a 20  $\mu$ l reaction volume containing 5  $\mu$ l template DNA, 4.5  $\mu$ l purified water, 10  $\mu$ l TaqMan Universal PCR Master Mix (2X) and 0.5  $\mu$ l of each SNP mix (40X). SNP genotyping was performed using standard TaqMan SNP assays (ABI, Foster City, CA) carried out by a 7500 Fast

Real-Time PCR Systems (Life Technologies, California, USA). PCR amplification program was as follow: 1 cycle of 95°C for 10 minutes, 40 cycles of 92°C for 15 seconds and 60°C for 1 minute.

### Statistical analysis

The data were processed and analyzed using SPSS software version 20.0. Version 3.25 of the Power-Marker software was used for the determination of the Hardy-Weinberg equilibrium and the calculation of allele and genotype frequencies. Changes were considered statistically significant at  $p < 0.05$ , using the Fisher Exact test. Odds ratio (OR) and confidence intervals (CI) at 95% were calculated to estimate the associations of HPV infection with the rs 1800629 and rs 1946518 polymorphisms using Epi Info 7.

**Ethical approval:** The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the Ethics Committee for Health Research of Burkina Faso (CERS) (Deliberation No. 2014-9-110).

**Informed consent:** Informed consent has been obtained from all individuals included in this study

## Results

### Participant demographics

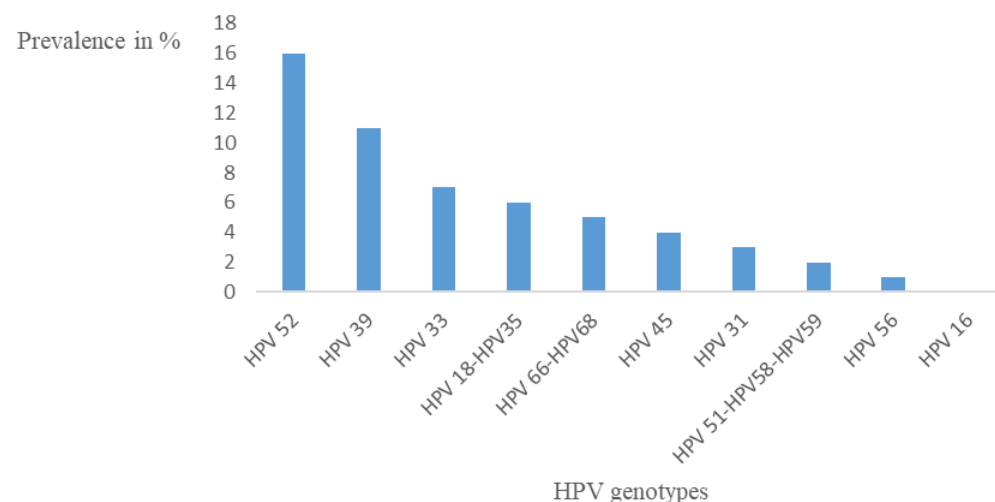
The characteristics of the two groups of women are given in Table 1. No statistically significant differences were found between cases and controls regarding age, age at first intercourse or number of pregnancies ( $p$  values were respectively 0.631; 0.103 and 0.513)

### Characteristics of HPV-HR infection in cases

Among the 91 patients infected with HPV-HR, 76.9% (70/91) were carriers of a single genotype of HPV; Multiple infections ranged from 2 to 4 genotypes. The most common genotype was HPV 52 (17.6%) followed by HPV 39 (12.9%) and HPV 33 (7.8%) (Figure 1).

**Table 1** : Demographic characteristics of cases and controls.

Groupes	n	Age (years)	Age at the first sexual intercourse	Number of pregnancies
Patients (HPV+)	91	34.9±9.4	17.9±2.5	3.6±2.7
Controls (HPV -)	209	34.7±8.6	18.3±2.9	3.5±2.4
P value	-	0.631	0.103	0.513

**Figure 1** : Frequencies of high-risk HPV genotypes found among women.

### Prevalence of TNF $\alpha$ (rs 1800629) and IL 18 (rs 1946518) polymorphisms

The allele and genotype frequencies of TNFA -308 (rs1800629) and IL18 -607 C/A (rs1946518) polymorphisms for cases and controls are listed in Table 2. Results demonstrated that the two SNPs rs 1800629 and rs 1946518 genotype are all in Hardy-Weinberg equilibrium (HWE).

The rs 1800629 A allele for TNF $\alpha$  was present in all women in the 2 study groups with a frequency of 84.61% and 84.69% respectively in cases and controls (Table II). None of the TNFA-308A or TNFA-308G alleles were associated with carrying HPV infection ( $p=0.92$ ).

The C allele of interleukin 18 was the most predominant in both cases (67.6%) and controls (61.5%). However, we have not found an association between the IL-607 C or IL-607 A allele and HPV infection in women.

## Discussion

The 3 most common HPV genotypes found in cases in this study was HPV 52, HPV 39 and HPV 33. In contrast,

HPV 16 has been found as the most common genotype in other studies [17, 18], yet HPV16 was not detected in our study. This result agrees with previous findings reported in Burkina Faso [19, 20] where HPV 16 and HPV 18 were not among the most frequent genotypes.

We investigated the distribution of allele and genotype of TNF $\alpha$ -308 (rs 1800629) and IL 18-607 (rs 1946518). The results showed that TNF $\alpha$ -308A was the predominant allele among all the women in the study. The frequency of this allele was 84.6% and 84.7% respectively in cases (women with positive HPV results) and controls (no HPV). There was no statistically significant difference for the A and G alleles of TNF  $\alpha$  ( $p=0.92$ ). It has been suggested that TNF  $\alpha$  may be able to directly fight against HPV infection by inducing apoptosis of virus-infected cells [21]. The frequencies of the TNF $\alpha$  -308 G/G and TNF $\alpha$  -308 A/A genotypes were not statistically different between cases and controls ( $p=0.6$  for G/G and  $p=0.8$  for AA). There was therefore no association between these genetic variants and susceptibility to HPV infection. In 2019, Chagas *et al.* [22] indicated in their study that the association of TNF $\alpha$  -308 represents a risk to the susceptibility in the development of cervical cancer in women infected by HPV

**Table 2:** Allele and genotype frequencies TNFA -308 (rs1800629) and IL18 -607 C/A (rs1946518) polymorphisms among women infected with HPV and negative HPV controls.

SNP	HPV – (Controls) n=209 (%)	HPV + (Cases) n= 91 (%)	OR (95% IC)	P value
<b>TNF<math>\alpha</math>-308</b>				
G/G	06 (02.9)	01 (01.1)	0.37 (0.04-3.17)	0.60
G/A	52 (24.9)	26 (28.6)	1.21 (0.69-2.09)	0.59
A/A	151 (72.2)	64 (70.3)	0.91 (0.53-1.56)	0.84
HWE*	0.84	0.65		
G	64 (15.3)	28 (15.4)	1.00 (Réf $\acute{e}$ rence)	0.92
A	354 (84.7)	154 (84.6)	0.99 (0.61-1.61)	0.92
<b>IL18-607</b>				
C/C	94 (45.0)	45 (49.4)	1.19 (0.72-1.96)	0.56
C/A	84 (40.2)	33 (36.3)	0.85 (0.51-1.41)	0.61
A/A	31 (14.8)	13 (14.3)	0.96 (0.47-1.93)	0.96
HWE*	0.25	0.26		
C	272 (65.1)	123 (67.6)	1.12 (0.78-1.62)	0.61
A	146 (34.9)	59 (32.4)	0.89 (0.62-1.29)	0.61

\* Hardy-Weinberg Equilibrium

58. Therefore, our results could be explained by the fact that this genotype was among the least frequent in the women of our study.

In Brazil, Tavares et al. had found that the TNF $\alpha$  -308 G/G genotype and the G allele were associated with increased susceptibility to HPV infection [14]. Meanwhile, it has also been reported that, the polymorphism of TNF $\alpha$  -308 G/A is associated with cancerous lesions. For example, in Zimbabwe, the TNF $\alpha$  -308 G/G genotype was more common in HPV positive women with cancer than in controls [23], while a study on Portuguese women with the A allele showed that those women were twice as likely to develop invasive cervical cancer [24]. Also, the authors of the Zimbabwe study found that the A allele as well as the A/A genotype were less common in their study than in populations in Europe. This is not the case for the women in our study. The A allele of TNF  $\alpha$  is known as TNF2, which would be associated with an increase in the production of this cytokine [9] and the level of TNF  $\alpha$  production in an individual would depend on his genetic predispositions [23].

Concerning the IL18 gene, the allele C was most common in women in both cases (67.6%) and controls (65.1%). Similarly, the predominant genotype was -607 CC in both cases (49.4%) and controls (45.0%). However, there were no significant differences in the frequencies obtained between the genetic variants -607 CC (p=0.56) and -607 AA (p=0.96) for cases and controls. None of these genetic variants therefore had an influence on HPV infection in our study. Tavares et al. [14] reported in their 2016 study that -607 C/A polymorphism in the

IL18 promoter was associated with protection against HPV infection. According to these authors, the -607 A/A genotype provided protection against HPV infection but did not affect progression to cancerous lesions. However, the results of a meta-analysis showed that -607 C/A polymorphism was associated with the risk of developing all types of cancer [25]. This polymorphism leads to a decrease in IL18 production due to a deficiency in the immune response against HPV, which would increase the risk of infection.

It is possible that the expression levels of the TNF $\alpha$  and IL18 genes may partly affect the persistence of HPV infection [21] while it is linked to progression to cervical cancer. The effect of these genes on HPV infection appears to be influenced by the cytokine gene products. As the production level of these is genetically controlled, the results of one study will be true for a given population whereas they would be different in another.

To our knowledge this is the first study investigating the effects of these two genes TNFA and IL18 on HPV infection among women in Burkina Faso. The study demonstrated that TNF-308 G/A and IL-18 C/A SNPs were not associated with HPV infection in the studied population. The results can be useful to identify the host genetic factors of HPV-related diseases. But it would therefore be important to complete the genetic association studies with the characterization of the effects of these SNPs by measuring serum cytokine levels bigger population sample size.

**Conflict of interest:** Authors state no conflict of interest

## References

- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018 Nov;68(6):394–424.
- Burd EM. Human papillomavirus and cervical cancer. *Clin Microbiol Rev*. 2003 Jan;16(1):1–17.
- Chinchai T, Homchan K, Sopipong W, Chansaenroj J, Swangvaree S, Junyangdikul P, et al. Lack of Associations between TNF- $\alpha$  Polymorphisms and Cervical Cancer in Thai women. *Asian Pac J Cancer Prev*. 2016;17(3):953–6.
- Horiuchi T, Mitoma H, Harashima S, Tsukamoto H, Shimoda T. Transmembrane TNF- $\alpha$ : structure, function and interaction with anti-TNF agents. *Rheumatology (Oxford)*. 2010 Jul;49(7):1215–28.
- Rothe J, Lesslauer W, Lötscher H, Lang Y, Koebel P, Köntgen F, et al. Mice lacking the tumour necrosis factor receptor 1 are resistant to TNF-mediated toxicity but highly susceptible to infection by *Listeria monocytogenes*. *Nature*. 1993 Aug;364(6440):798–802.
- Loetscher H, Stueber D, Banner D, Mackay F, Lesslauer W. Human tumor necrosis factor alpha (TNF alpha) mutants with exclusive specificity for the 55-kDa or 75-kDa TNF receptors. *J Biol Chem*. 1993 Dec;268(35):26350–7.
- Abe Y, Yamauchi K, Kimura S. 75- but not 55-kDa tumor necrosis factor receptor is active in the homotypic aggregation and proliferation of human lymphokine-activated T killer (T-LAK) cells in vitro. *J Leukoc Biol*. 1995 Mar;57(3):462–8.
- Zheng L, Fisher G, Miller RE, Peschon J, Lynch DH, Lenardo MJ. Induction of apoptosis in mature T cells by tumour necrosis factor. *Nature*. 1995 Sep;377(6547):348–51.
- Kroeger KM, Carville KS, Abraham LJ. The -308 tumor necrosis factor- $\alpha$  promoter polymorphism effects transcription. *Molecular Immunology*. 4// 1997;34(5):391-399.
- Elahi MM, Asotra K, Matata BM, Mastana SS. Tumor necrosis factor alpha -308 gene locus promoter polymorphism: an analysis of association with health and disease. *Biochim Biophys Acta*. 2009 Mar;1792(3):163–72.
- Lebel-Binay S, Berger A, Zinzindohoué F, Cugnenc P, Thiounn N, Fridman WH, et al. Interleukin-18: biological properties and clinical implications. *Eur Cytokine Netw*. 2000 Mar;11(1):15–26.
- Puren AJ, Fantuzzi G, Gu Y, Su MS, Dinarello CA. Interleukin-18 (IFN $\gamma$ -inducing factor) induces IL-8 and IL-1 $\beta$  via TNF $\alpha$  production from non-CD14+ human blood mononuclear cells. *J Clin Invest*. 1998 Feb;101(3):711–21.
- Piskin IE, Karakas-Celik S, Calik M, Abuhandan M, Kolsal E, Genc GC, et al. Association of interleukin 18, interleukin 2, and tumor necrosis factor polymorphisms with subacute sclerosing panencephalitis. *DNA Cell Biol*. 2013 Jun;32(6):336–40.
- Tavares MC, de Lima Júnior SF, Coelho AV, Marques TR, de Araújo DH, Heráclio SA, et al. Tumor necrosis factor (TNF) alpha and interleukin (IL) 18 genes polymorphisms are correlated with susceptibility to HPV infection in patients with and without cervical intraepithelial lesion. *Ann Hum Biol*. 2016 May;43(3):261–8.
- Traore IM, Zohoncon TM, Dembele A, Djigma FW, Obiri-Yeboah D, Traore G, et al. Molecular Characterization of High-Risk Human Papillomavirus in Women in Bobo-Dioulasso, Burkina Faso. *BioMed Res Int*. 2016;2016:7092583–7092583.
- Traore IM, Zohoncon TM, Ndo O, Djigma FW, Obiri-Yeboah D, Compaore TR, et al. Oncogenic Human Papillomavirus Infection and Genotype Characterization among Women in Orodara, Western Burkina Faso. *Pak J Biol Sci*. 2016;19(7):306–11.
- Tjalma WA, Trinh XB, Rosenlund M, Makar AP, Kridelka F, Rosillon D, et al. A cross-sectional, multicentre, epidemiological study on human papillomavirus (HPV) type distribution in adult women diagnosed with invasive cervical cancer in Belgium. *Facts Views Vis Obgyn*. 2015;7(2):101–8.
- Monsonogo J, Cox JT, Behrens C, Sandri M, Franco EL, Yap PS, et al. Prevalence of high-risk human papilloma virus genotypes and associated risk of cervical precancerous lesions in a large U.S. screening population: data from the ATHENA trial. *Gynecol Oncol*. 2015 Apr;137(1):47–54.
- Ouedraogo RA, Zohoncon TM, Guigma SP, Angèle Traore IM, Ouattara AK, Ouedraogo M, et al. Oncogenic human papillomavirus infection and genotypes characterization among sexually active women in Tenkodogo at Burkina Faso, West Africa. *Papillomavirus Res*. 2018 Dec;6:22–6.
- Salambanga C, Zohoncon TM, Traoré IM, Ouedraogo RA, Djigma WF, Ouédraog C, et al. Forte prévalence de l'infection au papillomavirus humain (HPV) à haut risque chez les femmes sexuellement actives dans la ville de Ouagadougou, Burkina Faso. *Med Sante Trop*. 2019 Aug;29(3):302–5.
- Basile JR, Zacny V, Münger K. The cytokines tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and TNF-related apoptosis-inducing ligand differentially modulate proliferation and apoptotic pathways in human keratinocytes expressing the human papillomavirus-16 E7 oncoprotein. 2001;276(25):22522-22528.
- Chagas BS, Lima RC, Paiva Júnior SS, Silva RC, Cordeiro MN, Silva Neto JD, et al. Significant association between IL10-1082/-819 and TNF-308 haplotypes and the susceptibility to cervical carcinogenesis in women infected by Human papillomavirus. *Cytokine*. 2019 Jan;113:99–104.
- Stanczuk GA, Sibanda EN, Tswana SA, Bergstrom S. Polymorphism at the -308-promoter position of the tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) gene and cervical cancer. *Int J Gynecol Cancer*. 2003 Mar-Apr;13(2):148–53.
- Duarte I, Santos A, Sousa H, Catarino R, Pinto D, Matos A, et al. G-308A TNF- $\alpha$  polymorphism is associated with an increased risk of invasive cervical cancer. *Biochem Biophys Res Commun*. 2005 Aug;334(2):588–92.
- Yang X, Qiu MT, Hu JW, Jiang F, Li M, Wang J, et al. Association of interleukin-18 gene promoter -607 C>A and -137G>C polymorphisms with cancer risk: a meta-analysis of 26 studies. *PLoS One*. 2013 Sep;8(9):e73671.