

FAS-670 Gene Promoter Region Polymorphism in Cervical Intraepithelial Neoplasia Grade 3 (CIN 3) and Invasive Cervical Cancer

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Abstract

The human papillomavirus (HPV) is the etiologic agent of genital cancer, but alone it is incapable of inducing oncogenesis. Rather, progression to invasive lesions is associated with host immunity and interference in the process of cellular apoptosis. Among the several genes involved in cell death, the *FAS* gene appears to be an important factor. The aim of this study was to evaluate whether there is an association between polymorphisms of the *FAS*-670 gene promoter region and preinvasive and invasive lesions of the cervix. The *FAS* gene was evaluated for the presence of polymorphisms using polymerase chain reaction and restriction fragment length polymorphism techniques in 225 blood samples for the control group, as well as cervical tissue from patients with cervical intraepithelial neoplasia grade 3 (CIN 3; 75 cases) or invasive carcinoma (214 cases). The AG genotype of the *FAS*-670 gene promoter region was significantly more frequent in women with CIN 3, with an estimated risk of three times (OR = 3.0). No difference, however, was observed in the control group and women with cervical cancer. In women with cancer, the genotypes were similar in the different histological types and degree of tumor differentiation. Assessing allelic distribution (A or G), we observed no difference in frequency of genotypes in studied groups. These data suggest that polymorphism of the promoter region of the *FAS*-670 gene is associated with increased risk of CIN 3, but not for invasive cancer of the cervix.

Keywords

FAS, Genetic Polymorphism, CIN 3, Uterine Cervical Neoplasms, Apoptosis

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1. Introduction

Cervical cancer affects thousands of women around the world (471,000 cases per year) and is the second leading cause of death from cancer in women. Approximately 80% of cases occur in developing countries, where, in some regions, it is the most common form of cancer in women [1]. Brazil has one of the highest incidence rates of this neoplasia, reaching upwards of 50 - 83 cases per 100,000 women in some regions [1]. For the year 2014, the estimated incidence of cervical cancer was 18,503 new cases, and the estimated risk was 15.3 cases per 100,000 women, with the highest incidence being in the north of the country (23/100,000) [2].

The etiology of cervical cancer has been studied for over 150 years, and it is often associated with infection. With the recent advent of molecular biology, cervical cancer tumors were determined to arise partially from a sexually transmitted infectious agent, the human papillomavirus (HPV) [3]-[5]. This virus is present in more than 99.7% of cases of squamous-cell carcinoma of the cervix, and the remaining 0.3% may be associated with unknown type of HPV [6] [7].

Despite the high frequency of infection, the prevalence of intraepithelial lesions and cervical cancer is low. The natural history of HPV infection shows that most infections are transient (80%) and not associated with the detected cytological abnormalities [8]. Therefore, HPV alone is incapable of inducing cancer, although the reasons are poorly understood. One possible explanation is that other factors (cofactors) in addition to HPV are needed for the development of this neoplasm [9] [10]. For example, in addition to sexual activity, multiparity, chronic genital infections, hormonal and immunological alterations, other sexually transmitted diseases, contraceptives and smoking are important factors [8]. Possibly the most important factors are those that affect host immunity. Most of these cofactors alter the ability of the host to respond immunologically; thus, the host is more likely to be a chronic carrier of HPV, to show cellular transformation, or both [11].

The role of host genetic factors in cervical carcinogenesis is still poorly understood. Investigations are now focusing on new molecular markers associated with susceptibility to the development of cervical carcinoma, known as biomarkers of progression, such as loss of heterozygosity, FHIT, telomerase, DNA ploidy, PCNA, Ki-67, p16, Mcm and Cdc6. Recently, a polymorphism in the promoter region of the *FAS* gene was shown to be associated with cervical oncogenesis in some studies, [12]-[15] but not in others [16]-[20].

The gene for the *FAS* receptor (also known as APO-1, CD95 or TNFRSF6) with its ligand (*FASL*) plays an essential role in the physiological regulation of apoptosis [21] [22] and transmission of no apoptotic signals [23]. At least twenty *FAS* receptor gene mutations have been described in humans and may trigger pathogenesis of many human diseases [24].

In addition to mutations, genetic elements located in the promoter region, such as polymorphisms, which influence the transcriptional activity of this gene [16], affect expression of these genes. Huang et al identified two polymorphisms in the *FAS* promoter region, which were A/G substitutions at position 670 and 1377 of the amplifier region [25]. The presence of the *FAS*-670 promoter region A/G polymorphism has been described in several diseases, such as rheumatoid arthritis, systemic lupus erythematosus, [26] multiple sclerosis, [27] lung carcinoma, [28] [29] and carcinoma of the nasopharynx, [30] among others. However, few studies have shown an association between *FAS* gene polymorphisms and cervical carcinogenesis [12] [14]-[16] [20].

The aims of this study were to evaluate the *FAS* gene promoter region polymorphism at position-670 in blood samples (control group) and in cervical tissue with precancerous lesions (cervical intraepithelial neoplasia grade 3-CIN 3) and invasive cervical carcinoma to determine whether this polymorphism was associated with the pathogenesis of cervical carcinoma.

2. Materials and Methods

2.1. Patients

This study was observational and case-controlled. The sample size was calculated with Epi-Info 6.04 software based on reports of *FAS* gene polymorphisms being present in 90% of CIN 3 cases, 82% of cervical cancer cases and 70% in women without these diseases [13] [31].

We evaluated 514 cases, including 225 cases of women with normal cytology and colposcopy (control), 75 histologically proven cases of CIN 3 and 214 cases of invasive cervical carcinoma. These cases were observed on the Gynecology and Obstetrics Service of the University Hospital at the Federal University of Santa Catarina (UFSC) in Florianopolis, the Alfredo Abraão Cancer Hospital in Campo Grande, São Paulo Hospital of Escola Paulista de Medicina in São Paulo and the Hospital and Maternity Leonor Mendes de Barros in São Paulo, Brazil.

The exclusion criteria for the control group included women who were previously subjected to anticancer treatment (radiotherapy or chemotherapy) for any type of cancer. For the CIN 3 group, we excluded cases associated with invasive or micro invasive lesions, and for the invasive cancer group, we excluded cases of cervical carcinoma in which the primary lesion site was doubtful, cases with possible endometrial or adjacent-organ carcinoma (bladder or rectum) with involvement of the cervix and cervical carcinoma cases that was previously treated with radiotherapy and/or chemotherapy.

Women were divided by age into ≤ 50 years old and > 50 years old for the purpose of dividing women by menopausal status; the average age of menopause among Brazilian women is approximately 50 years old [32]. Other factors evaluated were ethnicity (white, black and Asian), the histological type of carcinoma (squamous carcinoma or adenocarcinoma) and the tumor grade (G1 for well differentiated cervical tumors, G2 for moderately differentiated tumors and G3 for poorly differentiated or undifferentiated tumors). For calculation of risk estimates, we grouped grades G1 and G2, which were considered less aggressive, and compared them with G3, which was regarded as more aggressive [33].

2.2. Procedures

The presence of the *FAS* gene polymorphism was determined using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) after DNA extraction of paraffined tissue and blood.

2.2.1. DNA Extraction: Paraffin Block (Technical Formaldehyde/Chloroform)

The most representative paraffin blocks from the CIN 3 group and the cervical carcinoma group were selected for the study. Small fragments of each block were washed three times with 1 mL xylene for 10 minutes each in a 2-mL Eppendorf vial. The material was then agitated at 200 revolutions per minute (rpm) at 37°C for 30 minutes. Samples were then washed twice with 1 mL of absolute ethanol, then washed with 1 mL of 50% ethanol and finally washed with 1 mL of autoclaved MilliQ water. Between each wash, the vials were shaken at 200 rpm for 30 minutes at 37°C and centrifuged at 13,000 rpm for 1 minute, and the supernatant was discarded. Then, 200 μ L of digestion buffer was combined with 10 μ L of proteinase K and heated to 37°C overnight or for 48 hours. This material was then centrifuged at 13,000 rpm for 1 minute. An equal volume of the DNA product GFX[®] was added to the supernatant and centrifuged at 13,000 rpm for 10 minutes. Then, 200 μ L of supernatant was placed in a new 1.5 mL Eppendorf vial, and 950 μ L of absolute ethanol and 20 μ L of 3 M sodium acetate (pH 5.2) were added. This solution was kept at -20°C for 24 hours and subsequently centrifuged at 13,000 rpm for 20 minutes to form a DNA pellet, which was washed with 80% ethanol and centrifuged at 13,000 rpm for 2 minutes twice. The residue of this process was dried at room temperature for 10 minutes and rehydrated in 100 μ L of autoclaved MilliQ water. At that point, the DNA was ready to be used in other reactions.

2.2.2. DNA Extraction: Blood (According to the GFX Kit[®])

For blood samples, genomic DNA was extracted using the GFX[®] kit for blood cells (Amersham-Pharmacia). A total of 100 μ L of whole blood was supplemented with 500 μ L of extraction solution, and the mixture was kept at rest for 5 minutes. Then, the mixture was added to a column filter and centrifuged at 8000 rpm for 1 minute. Then, 500 μ L of the wash solution was added, the product was centrifuged at 14,000 rpm for 3 minutes, and 100 μ L of autoclaved MilliQ water was added, preheated to 70°C and centrifuged at 14,000 rpm for 1 minute in a new Eppendorf vial. DNA was then ready to be used in other reactions.

2.2.3. DNA Amplification by PCR

To investigate the *FAS* gene polymorphism of the promoter region (amino acid position-670), we used the technique described by Rudert *et al.* [34] and Wang *et al.* [29], with the following primers to amplify the DNA fragment: forward: 5'-CTA CCT AAG AGC TAT CTA CCG TTC-3', reverse: 5'-GGC TGT CCA TGT TGT GGC TGC-3'.

2.2.4. Identification of *FAS* Gene Polymorphism by Restriction Enzyme Digestion Using RFLP

The restriction enzyme MvaI cuts DNA at specific sites. When the mutation creates a new restriction site, the fragments generated are smaller than the fragments of non-mutated DNA. These fragments are then separated by

the molecular weight through gel electrophoresis [35].

The wild-type *FAS gene* (A/A) showed two bands by agarose gel electrophoresis: a 233-base pair fragment and a 98-base pair fragment. This classification is considered less associated with diseases. In cases of heterozygosity (genotype A/G), the amplification and digestion of the *FAS gene* produced four bands of 233, 189, 98 and 44 base pairs. This classification is considered the most associated with disease. In cases of G/G mutation (*FAS* mutant), amplification and digestion with MvaI produced bands of 189, 98 and 44 base pairs. In all three conditions, the total number of base pairs involved is 331.

Identification of the polymorphism begins with fragmentation of a 10 μ L aliquot of PCR product by MvaI for approximately 4 hours or overnight at 37°C. Next, the solution is subjected to gel electrophoresis using 2% agarose gels with ethidium bromide staining, sorting the *Fas gene* according to the base pairs identified.

2.3. Statistical Analyses

Data were analyzed using Statistical Package for Social Sciences (SPSS; version 16.0, IBM®). To compare qualitative variables or frequencies in the form of proportions, we used Fisher's exact test. To compare quantitative age data, we initially used the Shapiro-Wilk test to verify that the data showed a normal distribution. With a p value of <0.05 ($\text{sig} = 0.003$), the age variable did not show a normal distribution, so we used the Kruskal-Wallis and Mann-Whitney tests. The expected genotype frequencies (AA, AG, and GG) were estimated from the observed allele frequencies, and deviations from Hardy-Weinberg equilibrium were calculated using a chi-square test. For all tests, we considered levels of 5% statistically significant ($p < 0.05$). For estimation of ratio values (odds ratio [OR]) between the case and control genotypes, the unconditional logistic regression test was performed with a 95% confidence interval [36].

2.4. Ethical Standards

The research project was approved by the Federal University of Santa Catarina (opinion 057/07) and Escola Paulista de Medicina (opinion 0256/07) Ethics in Human Research Committee.

3. Results

3.1. Demographic Feature of Study Population

Age and *FAS gene* polymorphism variables were assessed in all 514 cases. However, the ethnicity variable could only be evaluated in approximately half of the cases (247 cases, divided into 3 groups: white, black and Asian). Of the 214 cases of carcinoma of the cervix, we only had access to the histological type and degree of tumor differentiation in approximately half of the cases (123). Analysis of these data is presented in **Table 1**.

The age distribution was very similar between the control group and the carcinoma group, with approximately half of the cases being women less than 50 years old. However, significantly more women from the CIN 3 group were younger than 50 years of age ($>90\%$), (**Table 1**). Furthermore, the mean age was significantly different between the three groups ($p < 0.05$). Specifically, the CIN 3 case group (mean age 36.91 years old) was significantly younger than the control group (mean age 46.87 years old), which was significantly younger than the cancer group (mean age 51.64 years old).

In evaluating the ethnicity variable, we observed a statistically significant difference among the three groups ($p < 0.05$). Specifically, while there was no significant between the control group and the CIN 3 group ethnicity ($p = 0.36$), the predominance of Caucasians among the cancer group (80%) was significantly different from controls.

Of the 123 cases with a known histological type of cancer, the vast majority (112 cases) had the squamous carcinoma type (91.1%), and the remainder (11 cases) had the adenocarcinoma type (8.9%).

3.2. Polymorphism Analysis

In analyzing the observed and expected genotype distribution of *FAS* polymorphisms in all three groups, we found that the CIN 3 group did not meet Hardy-Weinberg equilibrium ($p < 0.05$) but the control and cancer groups did ($p > 0.05$).

Table 1. Distribution of demographic and clinical variables assessed in the study.

Variable Category	Control		CIN 3		Carcinoma		P*
	n	(%)	n	(%)	n	(%)	
Age (N = 514)							<0.001
• <50 Y	130	(57.8)	69	(92.0)	110	(51.4)	
• >50 Y	95	(42.2)	06	(08.0)	104	(48.6)	
Total	225	(100)	75	(100)	214	(100)	
Ethnicity (N = 247)							<0.001
• White	20	(40.8)	38	(50.7)	98	(79.7)	
• Black	29	(59.2)	37	(49.3)	21	(17.1)	
• Asian	0	(0)	0	(0)	04	(03.3)	
Total	49	(100)	75	(100)	123	(100)	
Histological Type (N = 214)							
• Squamous carcinoma					112	(91.1)	
• Adenocarcinoma					11	(08.9)	
• Unknown					91		
Total					214	(100)	
Gene FAS Polymorphism (N = 514)							0.046
• Wild (AA)	53	(23.5)	08	(10.7)	52	(24.3)	
• Heterozygote (AG)	112	(49.8)	50	(66.7)	104	(48.6)	
• Mutant (GG)	60	(26.7)	17	(22.6)	58	(27.1)	
Total	225	(100)	75	(100)	214	(100)	

*Fisher exact test.

We evaluated the distribution of genotypes of the promoter region of the *FAS-670 gene* in the three groups, and we observed a statistically significant difference between them ($p < 0.05$). Specifically, the CIN 3 group had a statistically significant higher prevalence of the AG genotype (66.7%) (Figure 1). However, we found no significant difference between the control group and the cancer group in genetic composition of the promoter region ($p > 0.05$) (Table 2).

When we stratified age, we found that there was no association between genotype and increased risk for disease in either age group (≤ 50 and > 50 years old). In all three groups, the heterozygous genotype (AG) was the most frequent.

When comparing the genotypes of the *FAS gene* with known histological types of cervical carcinoma cases, we found no statistical difference between them, and the heterozygous genotype (AG) remained the most frequent, approximately 40% for both squamous carcinoma and adenocarcinoma.

3.3. Allele Analysis

We assessed the allelic distribution (A and G) of the promoter region of the *FAS-670 gene* in the three groups and found no significant difference ($p > 0.05$) (Table 3).

3.4. Estimates of Independent Risk Factors (OR)

Because the OR is an estimate of risk for developing the disease under study, we conducted a logistic regression analysis using the AA genotype as a reference. We included risk factors that were significant from individual case reviews.

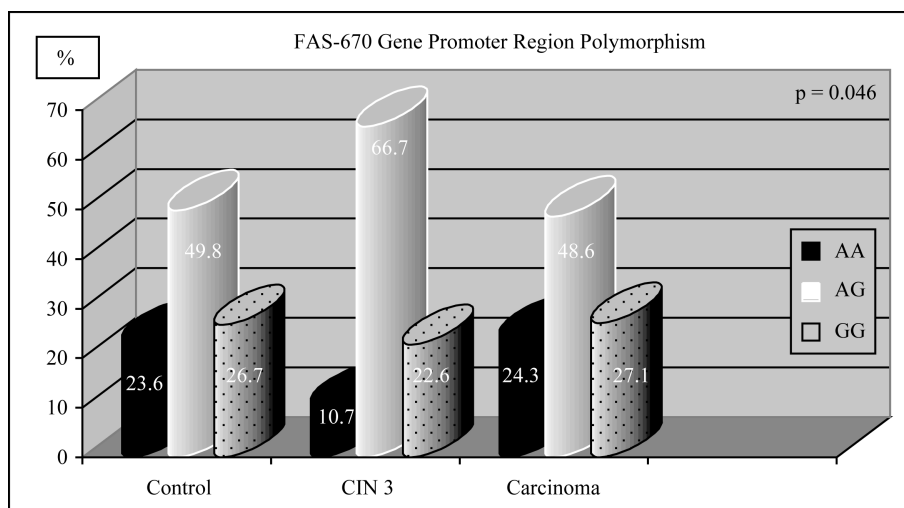


Figure 1. Polymorphism frequency of the *FAS-670* promoter region in the studied groups.

Table 2. Genotypic frequency of the *FAS-670* polymorphism in the studied groups.

Status	Polymorphism						Total	Fisher exact test	
	AA		AG		GG				
	n	(%)	n	(%)	n	(%)			
Control	53	(23.6)	112	(49.8)	60	(26.7)	225	100.0	0.046
CIN 3	08	(10.7)	50	(66.7)	17	(22.6)	75	100.0	
Cancer	52	(24.3)	104	(48.6)	58	(27.1)	214	100.0	
Total	113	(22.0)	266	(51.8)	135	(26.3)	514	100.0	

Table 3. Distribution of allelic polymorphism of the *FAS-670* gene in the studied groups.

Status	Allele				Total	Fisher exact test	
	A		G				
	n	(%)	n	(%)			
Control	218	(48.4)	232	(51.6)	450	100.0	0.60
CIN 3	66	(44.0)	84	(56.0)	150	100.0	
Cancer	208	(48.6)	220	(51.4)	428	100.0	
Total	492	(47.9)	536	(52.1)	1028	100.0	

Comparison of quantitative variables between the control group and the CIN 3 group revealed that only age was significantly different between groups upon individual analysis. The unadjusted risk estimate of genotype AG for the CIN 3 group was almost three times higher than the risk estimate for controls (OR = 2.96; 95% CI = 1.31 - 6.68; $p = 0.01$). Furthermore, this risk effect of the AG genotype remained even after adjusting for age (OR = 3.04; 95% CI = 1.29 - 7.16; $p = 0.01$), suggesting that this variable was not a confounding, moderating or mediating factor in the relationship between the polymorphism and the disease under study.

Individual case analysis of the control group and the cancer group revealed that age and ethnicity were different between the groups. Analysis of unadjusted risk revealed that no genotype influenced cervical cancer ($p > 0.05$). Furthermore, inclusion of age and ethnicity variables did not change the results, suggesting that these variables were not confounding, moderating or mediating factors of the relationship between polymorphism and

cervical cancer, *i.e.*, the genotype had no influence on cancer ($p > 0.05$) (**Table 4**).

We also analyzed cases of carcinoma, grouping women by age into ≤ 50 years and > 50 years. Using the AA genotype as the reference value, we found that no genotype of the promoter region of the *FAS-670 gene* had any greater influence on cancer risk, independent of age.

Finally, we assessed cases of carcinoma using squamous carcinoma as a benchmark, and we found that no genotype of the *FAS-670 gene* influenced the histological type. Furthermore, using the tumor grades G1 and G2 as benchmarks, we found that no genotype was associated with increased tumor aggressiveness.

4. Discussion

Cervical cancer is still one of the most common causes of cancer and death among women in developing countries. Although the role of high-risk HPV in cervical carcinogenesis is evident, only a small proportion of these cases progress to an invasive form of the disease [37], indicating that the development of cervical carcinoma is influenced by other factors, likely including genetic factors [19]. Decreased expression of the *FAS gene* and/or increased expression of its ligand (*FASL*) have been demonstrated in many types of human cancer and appear to be associated with human carcinogenesis and more aggressive tumors [38] [39]. Several factors must be considered before addressing the role of *FAS gene* polymorphisms in cervical cancer susceptibility. First, we should consider the geographical location and ethnic group under study. Although the AA genotype is considered normal [25], we can see in **Table 4** that distribution of the *FAS-670 gene* polymorphism is similar among the normal population in different countries, with the AG genotype (heterozygote) being the most commonly found (in approximately half the cases), followed by AA (wild-type) and finally GG (mutated) [12] [16] [19] [25] [27] [30] [40]-[43], just as we found in our study. In contrast, cases in Asian countries showed a much lower frequency of the GG genotype than other countries.

Individual phenotypic variations are often the result of slight differences in the sequence in the coding region

Table 4. A/G polymorphism distribution of the *FAS gene* at position-670 in the population of different geographical regions.

Author/Year	Country	Genotype (%)		
		AA	AG	GG
Nelson, 2001	USA	30.8	46.0	23.2
Huang, 1997	Australia	23.5	51.7	24.7
Niino, 2002	Japan	27.3	52.1	20.7
Seo, 2002	Korea	34.9	48.6	16.5
Lai, 2003	Taiwan	26.0	53.7	20.3
Dybikowska, 2004	Poland	29.2	52.3	18.4
Lai, 2005	Taiwan	28.6	50.6	20.8
Sun, 2005	China	43.6	44.2	12.2
Ueda, 2005*	Japan	30.2	-	-
Ueda, 2006	Japan	24.2	56.8	18.9
Jrad, 2006	Tunisia	31.2	50.8	17.8
Park, 2006	Korea	27.8	52.8	19.4
Kang, 2008	Korea	35.4	51.9	12.7
Mean		30.2	50.9	18.8
Present study	Brazil	23.5	49.8	26.7

*AG + GG: 69.8%; Dybikowska *et al.* Int J Mol Med 2004; 14(3): 475-8; Lai *et al.* Gynecol Oncol 2005; 99: 113-8; Ueda *et al.* Int J Gynecol Cancer 2006; 16: 179-82; Kang *et al.* Cancer Gene Cytogen 2008; 180: 1-5.

of the gene responsible for this phenotypic trait [25]. For example, variation in the promoter region of the *FAS* gene (position-670) may lead to different levels of expression of this gene. In addition, changes in the expression of the *FAS* gene can result in loss of or decrease in apoptotic function and, consequently, development of a disease or tumor [16]. This polymorphism has been studied in many diseases of infectious, immune or tumoral origin [26] [31] [38] [44]-[49]. More recently, studies have shown association between the polymorphism and preinvasive and invasive diseases of the cervix [12] [14]-[17] [20] [31] [42] [50]. In this study, we analyzed the *FAS*-670 gene promoter region polymorphism as a potential genetic marker for cervical carcinogenesis. Analysis of genotypes of this gene in cases of precancerous lesions, such as CIN 3, revealed a significant difference in the prevalence of genotype AG in cases with an adjusted risk estimate of three-fold. Similarly, Ueda *et al.* examined cervical smears and noted that the G allele frequency increased from low-grade lesions to high-grade lesions (specifically, high-grade cervical squamous intraepithelial lesion [HSIL]) and that there was an increased OR for the G allele in HSIL compared with the control group [31]. The authors concluded that this increased frequency of AG or GG genotypes in HSIL could result in a significant reduction in the expression of the *FAS* gene and a subsequent decrease in apoptosis of cells with injury. Furthermore, Lai *et al.* reported a significant difference in the AA genotype in the HSIL and cancer group compared with the control group (AA: 38% vs. 28.6%, respectively), with an estimated risk of 1.7 [42].

Because HPV infection with high oncogenic risk is inversely correlated with apoptosis of cervical epithelial cells, it is relevant to suggest that the presence of the *FAS*-670 G allele, which is associated with a lower expression of *FAS* efficiency, may be a risk factor for cervical carcinogenesis [19]. However, studies investigating the *FAS* gene and cervical cancer have been very controversial. For example, one study in a Taiwanese population showed that the *FAS*-670 A allele was associated with an increased risk of squamous intraepithelial lesions and squamous-cell carcinoma [12]. However, Ueda *et al.* showed that the GG genotype was associated with an increased risk of cervical cancer in the Japanese population [13]. Contrary to these two studies, five other studies [16]-[20] reported that the *FAS*-670 polymorphism was not associated with an increased risk of cervical carcinoma, similar to our findings. Comparison of the association between the *FAS*-670 polymorphism and cervical cancer is presented in Figure 2. The risk to develop cervical cancer resulted from this combined analysis was not increased (OR = 1.28; 95% CI = 0.69 - 1.87; $p > 0.05$). However, the tumor histologic type should be considered, despite the small number of no squamous cervical carcinoma in all studies. Zoodma *et al.* observed that the *FAS* polymorphism was weakly associated with adenocarcinoma of the cervix but not epidermoid carcinoma [18]. Our results showed no association between any *FAS* genotype and the histological type or degree of tumor differentiation. Similarly, other authors found no significant association between an increased risk of cervical carcinoma, advanced stage or histological type and polymorphisms in the *FAS* gene [16] [19] [20]. Sun *et al.*

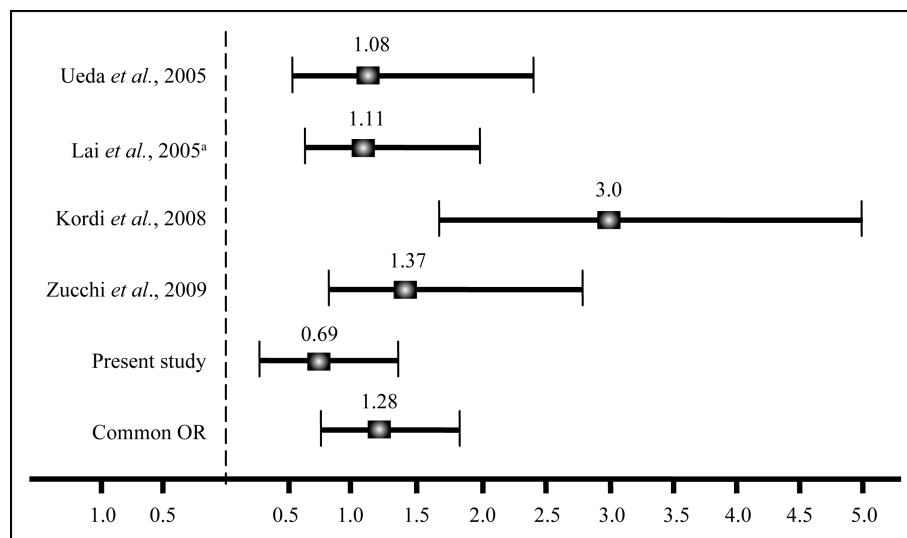


Figure 2. The association between *FAS*-670 gene polymorphism and cervical cancer. Data are reported odds ratio and 95% confidence interval for the presence of the AG genotype. ^aLai *et al.*, 2005: HSIL + squamous cell carcinoma.

assessed risk of cervical cancer among Chinese women with *FAS* and *FASL* polymorphisms and found that only the *FASL* -844 *gene* polymorphism (*i.e.*, switching of C to T) was associated with an increased risk of cancer [51]. Differences in samples, techniques and genomic variation of the *FAS*-670 *gene* among different ethnicities may explain the discrepancy of these results.

HPV infection is a necessary condition for genital cancer. However, HPV needs to be coupled with other factors for oncogenesis to occur [52] [53]. Among these factors, the most important are smoking, multiparity, immunodeficiency, diet and other concomitant infections (mainly *Chlamydia trachomatis* and herpes simplex 2). Polymorphisms of the *FAS* and *FASL genes* may increase the risk of cancer by interacting with these environmental factors. For example, Zhang *et al.* reported a significant interaction between *FAS gene* polymorphisms and smoking and lung cancer [54]. In addition, Sun *et al.* observed that the polymorphisms of the *FAS* and *FASL genes*' promoter regions were associated with the development of esophageal squamous-cell carcinoma in a Chinese population of tobacco users [55]. The fact that smoking may increase expression of *FASL* [56] [57] and increase susceptibility to lung cancer and esophageal cancer suggests that this gene-environment interaction may have important implications for the development of human cancer related to tobacco [54]. However, Park *et al.* evaluated three different types of gene polymorphisms of *FAS* and *FASL* in a Korean population and found no association, either alone or in combination, with other risk factors of lung cancer [43]. Several studies in recent years have shown a correlation between smoking and cervical cancer [8] [10] [58] [59] while others have not [9] [60] [61]. The Multicenter Study Program of Cervical Cancer of the International Agency for Research on Cancer (IARC) reviewed the evidence concerning smoking and cervical cancer and concluded that smoking is an independent risk factor for this type of cancer [62]. Mechanisms associated with cervical carcinogenesis and smoking include inhibition of apoptosis by nicotine leading to tumor growth [10] [63] and increased expression of *FAS* and *FASL genes* on lymphocytes by metabolites of smoking, which increases lymphocyte apoptosis [57]. However, no studies have assessed the association between smoking, polymorphisms of the *FAS gene* and cervical cancer, except Kordi *et al.*, which observed high risk of cervical cancer in passive smokers with AG genotype (OR = 4.6) [14].

Another cofactor that could interact with the *FAS gene* is hormones. As for tobacco, the IARC Multicenter Study Program of Cervical Cancer has demonstrated that the use of oral contraceptives for more than five years significantly increases the risk of cervical cancer (OR = 2.72), especially after 10 years (OR = 4.48) [62]. Estrogen can trigger apoptosis of T cells by increasing expression of *FAS* and *FASL* in cancer cells. Using a nonclassical mechanism, the estrogen receptor can regulate this expression by interacting with other substances [42] [64]. In our study, we attempted to assess the hormonal factor using menopausal status by separating women into age groups of greater or less than 50 years of age, which is the average menopause age among Brazilian women [32]. However, the age factor was not correlated with the frequency of *FAS* genotypes or the increased risk of cervical cancer.

Our study had some limitations. First, the sample size may have prevented us from finding an association between *FAS* polymorphisms and susceptibility to cervical cancer. Second, polymorphism of the *FAS gene* has a variable penetrance over time [65] and perhaps the age of the women may have interfered in these results, since we observed a significant association of genotype AG with CIN 3 (younger women) and not significant association with cancer (older women) compared to the control group. Third, the control group was not proportional to the cases in relation to the region, whereby the racial factor or mainly ancestry [66] could influence the results. Fourth, the HPV type was not assessed. It is possible that some specific type of HPV may suffer interference from any genotype of *FAS gene* and lead to the persistent infection and cervical oncogenesis. And finally, we did not include information on other exposure factors, which may alter the results and explain the conflict between our findings and those of several studies in the literature.

5. Conclusion

The authors conclude that the different genotypes of the *FAS gene* (position-670) promoter region are not associated with cervical carcinogenesis. However, our data suggest a possible relationship between increased risk of CIN 3 and the AG genotype, with risk being increased three-fold when adjusted for the age variable. Future studies should correlate the cofactors associated with carcinogenesis. These studies will show whether this polymorphism may be used as a genetic marker of susceptibility to the development of cervical cancer.

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