Modern Pathology (2009) 22, 1075–1082

www.modernpathology.org

HLA-G polymorphisms in women with squamous intraepithelial lesions harboring human papillomavirus

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Human papillomavirus (HPV) infection is etiologically associated with low- (LSIL) and high-grade squamous intraepithelial lesions (HSIL) and with cervical cancer. The progression or regression of the lesions may depend, among other factors, on the host heritable immune response. Because human leukocyte antigen (HLA)-G molecules are involved in the modulation of innate and adaptive immune responses, and because no previous studies have evaluated *HLA-G* polymorphism in patients with SIL, we conducted a study to assess the association between *HLA-G* polymorphisms and cervical lesions harboring HPV infection. Cervico-vaginal scrapings and blood samples were collected from 125 women with SIL (68 LSIL and 57 HSIL) and from 94 healthy women without HPV infection and cytological abnormalities. HPV type and HLA-G polymorphisms in exons 2, 3 and 8 (14 bp insertion/deletion) were evaluated by PCR methodology, and digested with restriction endonucleases. The Genepop software and the EM and PHASE algorithms were used for statistical analysis. A significant protective association was observed between the presence of the G*0103 allele and SIL and between the G0101/G0104 genotype and HSIL in the group of patients compared to control. The presence of the G0104/+14 bp and G0104/−14 bp haplotypes conferred susceptibility to SIL compared to control. In addition, patients possessing the G0104/+14 bp haplotype and harboring HPV-16 and -18 co-infections were particularly associated with HSIL. These findings suggest that *HLA-G* polymorphisms may be associated with HPV infection and SIL, consequently representing a profile of predisposition to cervical cancer.
Modern Pathology (2009) **22,** 1075–1082; doi:10.1038/modpathol.2009.67; published online 1 May 2009

Keywords: *HLA-G* gene polymorphism; *HLA-G* haplotypes; cervical cancer; human papillomavirus infection; HPV

Human papillomavirus (HPV) infects the mucosal and cutaneous epithelium causing benign warts and genital lesions. Low-risk HPV types are associated with the development of genital warts, and high-risk types such as HPV-16 and HPV-18 are associated with the development of low- and high-grade squamous intraepithelial lesions (LSIL and HSIL) and cervical cancer.[1] Although HPV infection is eliminated in 70–90% of cases, infected cells may develop mechanisms to escape from the immune response,[2] leading to persistent HPV infection and oncogenic transformation.[3]

The human major histocompatibility complex (MHC), known in humans as the human leukocyte antigen (HLA) system, is a highly polymorphic genomic region located on the 6p21.3 chromosome. Several functionally different MHC genes/molecules have been described such as classical HLA class Ia (*HLA-A, B, C*), nonclassical HLA class Ib (*HLA-E, -F, -G*), classical HLA class II (*HLA-DR, DQ, DP*) and

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Received 19 October 2008; revised 18 March 2009; accepted 30 March 2009; published online 1 May 2009

class III (complement system components and tumor necrosis factors, among others). The major function of classical HLA class Ia and class II molecules is antigen presentation to T CD8+ and T CD4+ cells, respectively, whereas nonclassical class Ib molecules are involved in the regulation of the immune response through interaction with surface receptors on natural killer (NK), T and antigen-presenting cells.[4] Several lines of evidence support the involvement of classical HLA molecules in the development of HPV-related cervical cancer. Some viral proteins, including the E7 protein of HPV-16 and HPV-18, may downregulate the cell-surface expression of classical HLA class I antigens,[5] allowing infected cells to escape from T CD8+ cytolytic cell killing. However, the lack of HLA classical class I expression may expose an infected cell to the attack of NK cells. On the other hand, the lytic action of NK may be regulated by the interaction of the specific NK immunoglobulin-like receptor (KIR) with class I HLA-C, -G and -E molecules.[6]

HLA-G has a restricted distribution on normal tissue cells, being primarily expressed in the thymus, pancreas and intestines. HLA-G is also abundantly expressed in placental tissue, particularly in the extravillous cytotrophoblast,[7] being implicated in the inhibition of the cytotoxic function of maternal NK cells.[8] The *HLA-G* gene generates multiple protein isoforms by alternative splicing of a single mRNA, giving rise to four membrane-bound isoforms (HLA-G1mb to -G4mb), and three soluble isoforms (HLA-G5s to -G7s) generated by the presence of a stop codon in intron 4.[9] HLA-G transcripts are also expressed at low levels in a variety of normal human adult tissues;[10] however, normal cervical cells do not express HLA-G.[11] HLA-G expression may be upregulated in inflammatory and neoplastic tissues,[12] and in viral infections.[13–15] The membrane-bound variant HLA-G1 suppresses the proliferation of T CD4+ cells, and the soluble variant HLA-G5 may induce apoptosis of activated T CD8+ cells.[16,17] Several polymorphic sites have been described for the *HLA-G* locus. Nucleotide substitutions mainly in exons 2, 3 and 4 may discriminate 42 confirmed alleles clustered into nine distinct allele groups, and generating only 15 different proteins due to the presence of several synonymous substitutions among *HLA-G* alleles (Anthony Nolan Research Institute, February 2009).[18] *HLA-G* alleles may influence the plasma levels of soluble HLA-G (sHLA-G).[19] In addition, a 14 bp insertion/deletion polymorphism has been reported in the 3′-untranslated region (UTR) of exon 8.[20] *HLA-G* alleles exhibiting the 14 bp insertion (+14 bp) undergo an alternative splicing that removes 92 bases from 3′ UTR,[21] influencing the stability of HLA-G mRNA.[22] Although removal of the 92 bases produces a more stable mRNA, the presence of +14 bp has been associated with a lower mRNA production.[20]

Some virus including Epstein–Barr virus, rabies virus and cytomegalovirus may upregulate HLA-G expression;[13,23–25] however, the function of HPV in HLA-G expression has not been determined. Recently, we reported that cervical lesions may express HLA-G molecules that may differ according to the severity of SIL in HPV-infected women. As the cervical lesion progressed from low to high grade, a decreased expression of HLA-G was observed.[11] Whether the expression of HLA-G may depend on HPV type, genetic background of patients or both is a question that has not been elucidated. In the present study we evaluated the polymorphism of the *HLA-G* locus, including the 14 bp insertion/deletion in exon 8, in patients harboring HPV infection, stratified according to the lesion severity and the presence of the most common oncogenic HPV types, ie, HPV-16 and -18.

Patients and methods

Study Design and Subjects

A total of 219 sexually active women were studied: 125 of them presented a cytological and colposcopic suspicion of HPV infection (patient group), and 94 had no cytological and/or colposcopic abnormalities (control group). Patients and controls were aged 15–71 years (median = 31.07 ± 10.9 and 34.05 ± 15.1, respectively), and were from the same geographic area, presumably presenting similar ethnic backgrounds. Although individuals that compose the Brazilian population are highly admixed, both patients (78.2%) and controls (82%) were primarily of European ancestry. Patients underwent routine gynecological examination at the Division of Infectious Diseases of the Department of Gynecology and Obstetrics, University Hospital, School of Medicine of Ribeirão Preto, University of São Paulo, Brazil. The local and the Brazilian Institutional Ethics Committees on human experimentation approved the study (protocols 1635/2002 and 885/2002, respectively). All patients gave written informed consent to participate in the study.

Sample Collection

Patients and controls were submitted to peripheral blood withdrawal and cervical scraping at the time of the medical visit. Because 10–30% of normal women may harbor HPV and do not exhibit SIL, and because this group may exhibit a transient infection, which may evolve to virus elimination or virus persistency (SIL), only those with no previous history of infectious diseases, without colposcopic or cytological abnormalities and exhibiting no DNA HPV detection were selected to form the control group. Patients were also submitted to cervical biopsies whenever indicated. Tissues were sectioned and evaluated histologically after staining

with hematoxylin and eosin, and two experienced histopathologists determined the lesion stage in a double-blind protocol.

To obtain DNA for HPV detection and typing, cervico-vaginal scrapings were collected with appropriate brushes and placed in microtubes containing the following buffer: 0.01 M Tris/HCL (pH 7.6), 5 mM $MgCl_2$ and 1% Triton X-100. DNA was extracted from peripheral blood and cervical scrapings as previously described.[26]

According to the results of cervical cytology, biopsy and DNA HPV detection, patients were stratified into two subgroups: 68 women with LSIL (cervical intraepithelial lesion grade I) and 57 with HSIL (cervical intraepithelial lesion grades II and III).

HPV Detection and Typing

MY09 and MY11 primers,[27] which amplify a 450 bp DNA fragment, were used for generic HPV amplification. Because HPV-16 and -18 are the most frequent types associated with SIL and invasive cervical lesions in Brazil,[28–30] attention was focused on them, using previously described specific primers and conditions.[31] The absence of amplification by HPV-16 or -18 primers but the presence of amplification by the MY09 and MY11 primers did not exclude the presence of other HPV types, which in this study were designated as *HPVX.* All reactions were performed using positive controls for HPV-16 (SiHa cell line) and HPV-18 (HeLa cell line) detection and controls for contamination.

HLA-G Typing

HLA-G polymorphism was defined by nucleotide sequence variations in exons 2 and 3. DNA amplification for *HLA-G* typing was carried out using previously described specific primers and cycling conditions.[32] Amplified DNA products were then digested with the restriction endonucleases *Hin*fI, *Hae*III, *Msp*I, *Bsp*1286I, *Bsr*BI for exon 2, and *Bse*GI, *Hga*I, *Cfr*13I for exon 3 (Fermentas Life Science, Burlington, Canada) according to the manufacturer's instructions. The cleavage products were submitted to 7% polyacrylamide gel electrophoresis and stained with silver nitrate.[33] The restriction fragment length polymorphism band pattern was used to define allele groups or specific alleles. Because most of the *HLA-G* alleles currently identified have synonymous substitutions, generating only nine different proteins when considering amino-acid sequences, and several of these groups are rare in the Brazilian population,[34] we primarily used allele groups, as described in Table 1, to correlate with SIL severity.

HLA-G 14 bp Polymorphism in Exon 8 (3′ UTR)

The *HLA-G* 14 bp polymorphism was typed as previously described.[35] The amplified products were visualized by electrophoresis on 4% Nusieve GTG agarose gel (Sigma, Saint Louis, MO, USA) stained with ethidium bromide (Sigma).

Table 1 Equivalence of the terminology for allelic groups and alleles

Allelic group	*Alleles*
G0101	G*01010101–G*010113, G*0106, G*0108, G*0109
G0102	G*0102
G0103	G*0103
G0104	G*010401–G*010404, G*0107
G0105N	G*0105N

HLA-G alleles recognized up to March 2008.

Statistical Analysis

The Genepop 3.4 software [36] was used to compare the frequencies of *HLA-G* allele groups. The associations between *HLA-G* polymorphisms among patients and controls, and among patients stratified according to lesion severity, HPV type or both, were evaluated by the two-sided Fisher's exact test and the odds ratio (OR), using the GraphPad InStat 3 software.[37] *P*-values were considered to be significant when ≤ 0.05.

The EM and PHASE algorithms [38,39] were used to determine *HLA-G* allele groups/14 bp polymorphism haplotypes. The Hardy–Weinberg equilibrium expectations were evaluated using the Genepop 3.4 software.[36]

Results

HPV Detection

All samples obtained from the patient group presenting a cytological and colposcopic suspicion of HPV infection were positive for HPV DNA by generic HPV amplification, and the samples obtained from the control group presented negative results for HPV DNA. The frequency (%) of HPV types in patients with LSIL and HSIL is described in Table 2.

HLA-G Alleles and the 14 bp Insertion/Deletion in Exon 8

The *HLA-G* genotypes did not fit the expected Hardy–Weinberg proportions in either the patient or control group ($P = 0.0040 \pm 0.0003$ and 0.0000 ± 0.0000, respectively), exhibiting heterozygote deficiency. The individuals included in the two groups were from different localities in São Paulo state, thus not representing a sample of a Mendelian population, a fact that may inflate the genetic diversity (expected heterozygosity) and lead to heterozygosis deficiency. However, the 14 bp

genotypes fit the Hardy–Weinberg expectations ($P = 0.1223 \pm 0.0015$ and 0.1667 ± 0.0012, respectively).

Four (G0101, G0103, G0104 and G0105N) of the five allelic groups evaluated in this study were observed. The G*0102 allele, known to be absent in the Brazilian population,[34] was not identified in this series of Brazilian subjects, Tables 3 and 4 show the frequency of the *HLA-G* allele groups and HPV genotypes observed in patients and controls. Table 5 illustrates the frequencies of *HLA-G*/14 bp haplotypes in patients and controls.

HLA-G in Patients and Controls

Considering the patient group irrespective of lesion severity and HPV type, the G*0103 allele was absent, being observed only in the control group (4.3%; Table 3). When patients and controls were compared, the G*0103 allele was found to be associated with protection against the occurrence of HPV lesions ($P = 0.0010$). The same protective effect was observed when we compared the presence of the G*0103/G*0103 genotype (Table 4). However, this was probably a 'hitch-hiking' effect of the above association, as all G*0103 individuals were homozygous for the allele (as confirmed by sequencing analysis). In addition, the G*0103/+ 14 bp haplotype was also shown to be protective (Table 5), although this is also the same effect of the G*0103 allele because this allele is known to be always associated with the presence of the 14 bp sequence in the Brazilian population.[34]

No significant results were observed when patients and controls were compared solely in terms of the presence or absence of the 14 bp polymorphism.

HLA-G in Patients Stratified according to Lesion Severity

The G0101/G0104 genotype was shown to be protective in patients presenting high-grade lesions when compared to LSIL patients, although with a borderline probability (OR = 0.2593, 95% CI = 0.06929–0.9701; $P = 0.0510$). When the HSIL group was compared to control, the same influence was found (OR = 0.2135, 95% CI = 0.06008–0.7584; $P = 0.0094$), indicating that the G0101/G0104 genotype may influence the susceptibility to high-grade lesions (Table 4).

HLA-G in Patients Stratified according to Lesion Severity and HPV Type

Compared with controls, the G0104/−14 bp haplotype was associated with low-grade lesions in women harboring *HPVX* (OR = 3.601, 95% CI = 1.080–12.012; $P = 0.0445$); however, no association was found when the XHSIL and XLSIL groups and the G0104 allele group itself were compared. The rare haplotype G0104/+14 bp[34] was only present in women with high-grade lesions harboring

Table 2 Distribution of HPV types in patients with LSIL and HSIL

	HPV types			
Lesion grade (n)	*HPV16,* n *(%)*	*HPV18,* n *(%)*	*HPV16/18,* n *(%)*	*HPVX,* n *(%)*
LSIL (68)	27 (51.9)	12 (80.0)	16 (57)	13 (43)
HSIL (57)	25 (48.1)	3 (20.0)	12 (43)	17 (57)
Total (125)	52 (41.6)	15 (12.0)	28 (22.4)	30 (24.0)

HPVX: unidentified HPV types; HPV: human papillomavirus.

Table 3 Distribution of *HLA-G* allelic groups and of the 14 bp polymorphism in controls (C) and patients (P), stratified according to lesion grade, HPV type and HPV type plus lesion grade

					Patients			
HLA-G	*Controls,* n *(%)*	*Patients,* n *(%)*	*Lesion grade,* n *(%)*		*HPV type,* n *(%)*			
Allelic group			*LSIL*	*HSIL*	*16*	*18*	*16/18*	*X*
G0101	147 (79.9)	212 (84.8)	114 (83.8)	98 (86)	92 (88.5)	27 (90)	46 (82.1)	47 (78.3)
G0103[a]	8 (4.3)	—	—	—	—	—	—	—
G0104	23 (12.5)	26 (10.4)	17 (12.5)	9 (7.9)	8 (7.7)	2 (6.7)	6 (10.7)	10 (16.7)
G0105N	6 (3.3)	12 (4.8)	5 (3.7)	7 (6.1)	4 (3.8)	1 (3.3)	4 (7.2)	3 (5)
Total	184	250	136	114	104	30	56	60
+14 bp	47 (39.8)	85 (43.8)	46 (45.1)	39 (42.4)	27 (36.5)	11 (50)	28 (56)	19 (39.6)
−14 bp	71 (60.2)	109 (56.2)	56 (54.9)	53 (57.6)	47 (63.5)	11 (50)	22 (44)	29 (60.4)
Total	118[b]	194[b]	102	92	74	22	50	48

LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion, *HPV*X: unidentified HPV types.
[a]Patients *vs* controls: $P = 0.0010$, OR = 0.04145, 95% CI = 0.002375–0.7233.
[b]Figures may not match as some samples could not be analyzed for the 14 bp polymorphism.

Table 4 Distribution of *HLA-G* genotypes in controls (C) and patients (P), stratified according to lesion grade, HPV type and HPV type plus lesion grade

			Patients, n *(%)*													
Genotypes	*Controls,* n *(%)*	*Patients,* n *(%)*	*LSIL*	*HSIL*	*16*	*18*	*16/18*	*X*	*16LSIL*	*16HSIL*	*18LSIL*	*18HSIL*	*16/18LSIL*	*16/18HSIL*	*XLSIL*	*XHSIL*
G0101/G0101	61 (66.3)	94 (75.2)	49 (72)	45 (79)	41 (78.8)	12 (80)	20 (71.4)	21 (70)	22 (81.5)	19 (76)	9 (75)	3 (100)	11 (68.8)	9 (75)	7 (53.8)	14 (82.3)
G*0103/G*0103	4 (4.3)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
G0101/G0104	19 (20.7)[a]	15 (12)	12 (17.6)[b]	3 (5.3)[a,b]	6 (11.5)	2 (13.3)	3 (10.7)	4 (13.4)	3 (11.1)	3 (12)	2 (16.7)	—	3 (18.8)	—	4 (30.8)	—
G0104/G0104	2 (2.2)	5 (4)	2 (3)	3 (5.3)	1 (2)	—	1 (3.6)	3 (10)	1 (3.7)	—	—	—	—	3 (8.3)	1 (7.7)	2 (11.8)
G0101/G0105N	6 (6.5)	9 (7.2)	4 (5.9)	5 (8.7)	4 (7.7)	1 (6.7)	3 (10.7)	1 (3.3)	1 (3.7)	3 (12)	1 (8.3)	—	1 (6.2)	2 (16.7)	1 (7.7)	—
G0104/G0105N	—	1 (0.8)	1 (1.5)	—	—	—	1 (3.6)	—	—	—	—	—	1 (6.2)	—	—	—
G0105N/G0105N	—	1 (0.8)	—	1 (1.7)	—	—	—	1 (3.3)	—	—	—	—	—	—	—	1 (5.9)
Total	92	125	68	57	52	15	28	30	27	25	12	3	16	12	13	17
+14 bp	12 (20.3)	24 (24.8)	12 (23.5)	12 (26.1)	7 (19)	3 (27.3)	9 (36)	5 (20.8)	3 (17.6)	4 (20)	2 (25)	1 (33.3)	5 (35.7)	4 (36.4)	2 (16.7)	3 (25)
+14 bp/−14 bp	23 (39)	37 (38.1)	22 (43.2)	15 (32.6)	13 (35)	5 (45.4)	10 (40)	9 (37.5)	6 (35.3)	7 (35)	3 (37.5)	2 (66.7)	7 (50)	3 (27.2)	6 (50)	3 (25)
−14 bp	24 (40.7)	36 (37.1)	17 (33.3)	19 (41.3)	17 (46)	3 (27.3)	6 (24)	10 (41.7)	8 (47.1)	9 (45)	3 (37.5)	—	2 (14.3)	4 (36.4)	4 (33.3)	6 (50)
Total	59	97	51	46	37	11	25	24	17	20	8	3	14	11	12	12

LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; *HPVX*: unidentified HPV types.
[a]HSIL *vs* control, $P = 0.0094$; OR = 0.2135, 95% CI = 0.06008–0.7584.
[b]HSIL *vs* LSIL, $P = 0.0510$, OR = 0.2593, 95% CI = 0.06929–0.9701.

Table 5 Distribution of *HLA-G* haplotypes in controls (C) and patients (P), stratified according to lesion grade, HPV type and HPV type plus lesion grade

			Patients													
	Controls, n *(%)*	*Patients,* n *(%)*	*Lesion grade,* n *(%)*		*HPV types,* n *(%)*				*HPV types and lesion grade,* n *(%)*							
Haplotype			*LSIL*	*HSIL*	*16*	*18*	*16/18*	*X*	*16LSIL*	*16HSIL*	*18LSIL*	*18HSIL*	*16/18LSIL*	*16/18HSIL*	*XLSIL*	*XHSIL*
G0101/+14 bp	35 (30.7)	63 (37)	33 (38.4)	30 (35.7)	24 (33.3)	9 (50.0)	15 (37.5)	15 (37.5)	11 (34.4)	13 (32.5)	6 (42.9)	3 (75.0)	10 (45.5)	5 (27.8)	6 (33.3)	9 (40.9)
G0101/−14 bp	56 (49.1)	79 (46.6)	37 (43.0)	42 (50.0)	40 (55.6)	6 (33.3)	18 (45.0)	15 (37.5)	18 (56.3)	22 (55.0)	5 (35.7)	1 (25.0)	8 (36.4)	10 (55.6)	6 (33.3)	9 (40.9)
G*0103/+14 bp	8 (7.0)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
G0104/+14 bp	—[a]	2 (1.2)	—	2 (2.4)	—	—	2 (5.0)	—	—	—	—	—	—	2 (11.1) [a]	—	—
G0104/−14 bp	11 (9.6)[b]	19 (11.2)	12 (14.0)	7 (8.3)	6 (8.3)	2 (11.1)	2 (5.0)	9 (22.5)	3 (9.4)	3 (7.5)	2 (14.3)	—	2 (9.1)	—	5 (27.8) [b]	4 (18.2)
G0105N/+14 bp	3 (2.6)	6 (3.5)	4 (4.7)	2 (2.4)	2 (2.8)	1 (5.6)	2 (5.0)	1 (2.5)	—	2 (5.0)	1 (7.1)	—	2 (9.1)	—	1 (5.6)	—
G0105N/−14 bp	1 (0.9)	1 (0.6)	—	1 (1.2)	—	—	1 (2.5)	—	—	—	—	—	—	1 (5.6)	—	—
Total	114	170	86	84	72	18	40	40	32	40	14	4	22	18	18	22

LSIL: low-grade squamous intraepithelial lesion; HSIL: high-grade squamous intraepithelial lesion; *HPV*X: unidentified HPV types; I/D 14 bp: insertion/deletion exon 8 14 bp.
[a]HSILHPV16/18 *vs* control: $P = 0.0177$; OR = 34.697, 95% CI = 1.594–755.46.
[b]LSIL*HPVX vs* control: $P = 0.0445$; OR = 3.601, 95% CI = 1.080–12.012.

both HPV-16/18 infections (OR = 34.697, 95% CI = 1.594–755.46; $P = 0.0177$) (Table 5); however, no association was found when LSIL 16/18 and HSIL 16/18 were compared.

Discussion

Several reports have evaluated HLA-G expression in fully developed cancer cells;[40] however, few studies have evaluated the expression of HLA-G in SIL or cervical cancer.[41–43] Polakova and Russ[43] studied different tumor cell types and observed no HLA-G expression on cervical cancer cells. Yoon *et al*[41] reported that high HLA-G mRNA expression was associated with early stage cervical cancer. In a previous study evaluating HLA-G and HLA-E expression in SIL biopsies, HLA-G expression was increased during the first stage of the lesions (LSIL), decreasing with increasing lesion grade (HSIL). In contrast, HLA-E expression exhibited an opposite behavior according to SIL severity.[11] The balance between constitutive HLA-G and virus-modulated expression may determine the outcome of the infection, facilitating or not virus persistence. This study was designed to evaluate the potential involvement of polymorphic sites of the *HLA-G* gene in the development of SIL in women harboring HPV infection.

Several *HLA-G* polymorphisms have been reported to be associated with differential expression of the HLA-G molecule.[19] In this study, the HLA-G*0103 was associated with protection against HPV lesions when patients considered as a whole were compared to controls. In addition, all the individuals presenting the G*0103 allele in this study were homozygous for this allele (confirmed by sequencing analysis), indicating that the associations with the G*0103 allele or G*0103/G*0103 genotype were not independent observations. The same protective effect was observed for the G*0103/+14 bp haplotype; however, this allele is known to always be associated with the insertion of the 14 bp fragment in the Brazilian population.[34]

Considering that the G*0103 allele ranks third in frequency (8.74%) in the normal Brazilian population,[34] and that in the present series it was absent in patients, these data indicate that this allele may protect against SIL even in the presence of the more oncogenic HPV types. Certain *HLA-G* alleles have been associated with the magnitude of HLA-G molecule production; however, no association between the G*0103 allele and levels of HLA-G production, HLA-G expression or both, has been reported.

On the other hand, the HLA-G*010401 allele has been associated with high plasma levels of sHLA-G.[19] Considering that the G*010401 and G*010404 alleles are the most frequent in the G0104 allele group, with a frequency of 8.25 and 3.88% in the normal Brazilian population, respectively,[34] and given that both alleles (1) have the same coding and non-coding sequences (except for a single nucleotide exchange in exon 4), (2) are related to the deletion of the 14 bp fragment,[34] (3) are probably associated with the same promoter activity and (4) probably have similar HLA-G production ability, the G0104 allele group, as well as the G*010401 allele, may be associated with high sHLA-G production.[19] In spite of a possible harmful influence of a high-producer allele group and SIL, in the present series the G0101/0104 genotype seemed to confer protection against the occurrence of HSIL.

In the present study, the G0104 group consisted of five different *HLA-G* alleles (Table 1), two of which have been previously associated with the deletion of the 14 bp fragment, and the others were rare in the Brazilian population and have no recognized 14 bp association.[34] However, the G*010401/+14 bp haplotype has already been reported, probably due to the occurrence of crossing-over inside the *HLA-G* gene or these may simply be haplotypes of African or Amerindian origin.[34] In the present series, the G0104/−14 bp haplotype seemed to be associated with low-grade lesions in women infected with *HPVX* compared to controls, but no association was found when low- and high-grade patients were compared. Similarly, the G0104/+14 bp haplotype was associated with high-grade lesions in women infected with both HPV-16/18 compared to controls, but no association was found when low- and high-grade patients were compared. Considering that the G0104 allele group is probably associated with a higher production of sHLA-G[19] and that the 14 bp insertion produces a more stable mRNA,[22] these findings indicate that women possessing the G0104/+14 bp haplotype may produce more HLA-G and, therefore, may evade the attack of NK cells. Although the strength of this association (OR) is high ($P = 0.0177$; OR = 34.697; 95% CI = 1.594–755.46), it should be noted that no association was found comparing LSIL and HSIL groups (only with controls), no individual association was found with the G0104 allele group (only when stratified into haplotypes), and the association between G0104/−14 and HSIL (16/18+) was based only on two positive cases (a rare haplotype in our population).

In this study, we reported that women presenting the HLA-G*0103 allele may be protected against SIL. In addition, women who harbor co-infection with HPV-16 and -18 and who possess the high-production *HLA-G*0104 allele group together with the presence of other mRNA stability factors, such as +14 bp, were at higher risk for the occurrence of HSIL compared with women without HPV infection and with women infected with other HPV types.

In conclusion, the G*0103 allele and G0101/G0104 genotype may be associated with protection against SIL. The G0104/+14 bp and G0104/−14 bp haplotypes conferred susceptibility to SIL compared to controls; however, patients possessing the G0104/+14 bp haplotype who presented HPV-16 and -18

co-infection were particularly associated with HSIL. Whether the amount of sHLA-G production and magnitude of HLA-G membrane expression or the balance between HLA molecules on the cervical cell surface that produce inhibitory or stimulatory signals for NK cells is necessary to modulate the outcome of the infection are questions that need to be further explored. Although other HLA class I and class II classical genes have been associated with SIL,[44–47] the results of the present study indicate that *HLA-G* polymorphisms as well as the HPV type are associated with SIL. To clarify the function of *HLA-G* alleles in SIL susceptibility, it would be interesting to conduct a longitudinal study on normal women exhibiting HPV infection with no SIL to determine whether these alleles are associated with elimination or persistence of HPV infection.

Acknowledgements

We thank Sandra Rodrigues and Ana Maria Rocha for excellent technical assistance and Dr Luisa Lina Villa for providing the HPV-16 and HPV-18 DNA-positive standard samples.

Financial Support: This work was supported by grants from CAPES (DS 103/00) to Simões, and from FAPESP (01/02908-2) and CAPES (0313/04-1) to Gonçalves (Brazilian and São Paulo State governments, respectively).

Disclosure/conflict of interest

This paper was reviewed and approved by all authors. There is no conflict of interests regarding this article.

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