Classical HLA alleles are associated with prevalent and persistent cervical high-risk HPV infection in African women

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A B S T R A C T

Background: Persistent cervical high-risk human papillomavirus (hrHPV) in-
fection is a necessary cause of cervical cancer. However, the host genetic factors underlying its risk are not well understood. We hypothesized that immunogenetic variation plays a role in hrHPV infection and persistence. Therefore, we conducted a study of classical HLA alleles and their association with hrHPV infection and persistence among women.

Methods: We characterized HPV infection using $SPF_{10}/LiPA_{25}$ in Nigerian women at baseline and at 6 months follow-up visits in 2014. hrHPV infection was prevalent if at least one carcinogenic HPV genotype was detected at the baseline visit and persistent if at least one carcinogenic HPV genotype was detected at the baseline and follow-up visits. Classical HLA alleles were imputed from genotypes in the MHC region using the HLA genotype imputation with attribute bagging (HIBAG) algorithm. HLA association tests were conducted under additive genetic models.

Results: The mean (± SD) age of the 517 study participants was 38 (± 8) years, 48% were HIV negative, 24% were hrHPV positive at baseline and 10% had persistent hrHPV infections. In multivariate regression models adjusted for age, HIV status and the first principal component, DQA1*01:02 and DQA1*02:01 were positively associated with prevalent but not persistent hrHPV infections, while DQA1*05:01 was negatively associated with prevalent hrHPV but positively associated with persistent cervical hrHPV infections. Four haplotypes (A*30:01-DQA1*05:01, B*07:02-C*07:02, B*07:02-DQA1*05:01 and C*07:02-DQA1*05:01) were significantly associated with prevalent cervical hrHPV infections and several haplotypes that included the DQA1*05:01 allelic variant were significantly associated with persistent cervical hrHPV infections. Six amino acid positions on DQα1 were associated with prevalent but not persistent cervical hrHPV infections.

Conclusions: In this first study to investigate the association between HLA alleles and persistent hrHPV in African women, we identified important risk alleles that merit further investigation. Our findings provide new insights into risk factors for hrHPV infection in African ancestry women.

1. Introduction

Persistent cervical infection with high-risk (hr) human papillomavirus (HPV) is necessary for the development of cervical cancer, the fourth leading cancer in women globally. In 2018, there were 569,847 incident cases and 311,365 deaths from cervical cancer globally [1]. While several behavioral and phenotypic cofactors for persistent cervical hrHPV infection have been identified, including cigarette smoking, multiple sexual partners and co-infection with HIV [2–4], the genetic factors associated with cervical hrHPV persistence are not well elucidated. It has been hypothesized that the human leucocyte antigen (HLA) complex, a set of genes involved in activating the cell-mediated immune response and identifying autologous and foreign proteins, is a cofactor for persistence of cervical hrHPV infections.

The HLA loci is the most polymorphic genetic loci in humans [5].

The highly polymorphic HLA molecules play a major role in immune response by binding and presenting foreign antigens, including HPV peptides, to T lymphocytes, thus promoting immune recognition and subsequent clearance of infected cells [6]. Individuals with HLA polymorphisms may produce proteins that have lower binding affinity to HPV antigens, are less likely to recognize cervical HPV infections, and thus have higher likelihood of associated persistent infections.

While most studies have focused on the associations between HLA and cervical cancer [7–16], few studies have examined the distribution of HLA alleles and their associated risk with hrHPV infections. One study among Canadian women showed that HLA-G alleles or genotypes were not significantly associated with persistence of alpha group 2 hrHPV or any HPV types [17]. On the other hand, Brazilian women who had cervical HPV infections were shown to be more likely to have DRB1*1503, DRB1-0405 and DQB1*0602 HLA alleles, compared to

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women without cervical HPV infections [18]. Till date, there are no previous studies on the association between HLA and cervical hrHPV infections among women in sub-Saharan Africa (SSA). In this study, we investigated the association between classical HLA alleles and cervical hrHPV infections, among African women.

2. Materials and methods

2.1. Study population

We examined 544 women from a cohort study of HPV infection and cervical cancer at National Hospital, Abuja and University of Abuja Teaching Hospital, Nigeria, who were enrolled between 2012 and 2014, as previously described [2,19–21]. All the study participants were 18 years or older, had a history of vaginal sexual intercourse, were not currently pregnant and had no history of cervical disease or hysterectomy. We collected data on socio-demographic characteristics, sexual and reproductive history, and self-reported HIV status of the participants. We confirmed participants' HIV status from hospital medical records. We collected venous blood samples and performed pelvic examinations on all study participants at each study visit. Elution swab system (Copan, Italy) was used to collect exfoliated cervical cells, which were inserted in 1 ml Amies' transport media (Copan). Participants were asked to return for follow-up after six months, at which time, the history, physical examinations and sample collections were repeated.

2.2. HPV detection by $SPF_{10}/LiPA_{25}$

We extracted DNA from the cervical exfoliated cells as previously described [22]. Samples were tested for HPV DNA by hybridization of SPF_{10} amplimers to a mixture of general HPV probes recognizing a broad range of high-risk, low-risk, and possible hrHPV genotypes in a microtiter plate format, as described previously [23]. All samples determined to be HPV DNA positive by SPF_{10} DNA Enzyme Immunoassay (DEIA) were genotyped using the $LiPA_{25}$ version 1. The $LiPA_{25}$ assay provides type-specific information for 25 different HPV genotypes simultaneously and identifies infection by one or more of 13 hrHPV genotypes: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 [24,25]. However, as this assay does not differentiate between HPV 68 and 73, we defined this HPV genotype (i.e. HPV68/73) as low-risk. We defined hrHPV infection as prevalent if at least one hrHPV genotype was detected in the baseline sample and persistent if at least one hrHPV genotype was detected in samples provided at both the baseline and follow-up visits. We defined persistently negative as absence of hrHPV genotype in the baseline or follow-up visit samples.

2.3. Statistical analysis

From the original set of 544 women, we excluded 27 women from the baseline analyses because of incomplete data (5 missing HPV, 22 missing both HPV and HIV results), leaving only 517 women in the baseline analyses. Of the 517 women, we excluded those who did not return for the follow-up visit (n = 9) and those with missing HPV results (n = 35) and included the remaining 473 women in the analyses for persistent hrHPV infections. To describe the baseline characteristics of the study population, we used *t*-tests to assess differences in the distribution of continuous variables, and χ^2 and Fisher's exact tests for categorical variables. Using dense SNP genotype data available on the same women, we computed principal components based on the variance-standardized relationship matrix using PLINK 1.9 [26,27]. We found that the first principal component was significant and included it in downstream association analyses.

2.4. HLA genotype imputation

Samples from the study participants were genotyped using the

Illumina Multi-Ethnic Global Array (MEGA). After technical quality control, sample-level genotype call rate was at least 0.95 for all the study participants. The HLA genotype imputation with attribute bagging (HIBAG) algorithm was used to derive high-resolution HLA types [28]. Attribute bagging is a technique for improving the accuracy and stability of classifier ensembles deduced using bootstrap aggregating and random subsets of variables [29]. HIBAG makes predictions by averaging HLA-type posterior probabilities over an ensemble of classifiers for European, Asian, Hispanic and African ancestries [28]. The prediction accuracies for HLA-A, B, C, DRB1 and DQB1 ranged from 92.2% to 98.1% [28].

2.5. Association analysis

Association analysis was done using PyHLA [30], a software package designed for association analysis of the HLA types imputed from genome-wide genotyping and next-generation sequencing (NGS) data. We used additive logistic regression models adjusted for age, HIV status and the first principal component, to test for associations between four-digit HLA allele and both prevalent and persistent cervical hrHPV infections. Fisher's exact tests and calculated odds ratio with Haldane's correction of Woolf's method were used to examine the association between HLA haplotype, amino acid sequence, allele and residual zygosity, and both prevalent and persistent cervical hrHPV infections. The zygosity tests evaluated the frequency difference of carrying the homozygous and heterozygous alleles and the absence of a particular allele or amino acid in women with hrHPV infections compared to those without hrHPV. When an allele or residual was associated (p < 0.05) with the disease, these tests were performed to identify whether a homozygote or heterozygote condition differentiates susceptibility to the disease. *p* values were adjusted for multiple comparisons with the false discovery rate (FDR) method.

2.6. Ethics

The study was conducted according to the Nigerian National Code for Health Research Ethics. Ethical approval to conduct this study was obtained from the Institute of Human Virology Nigeria research ethics committee. Written informed consent was obtained from all participants before enrollment in the study.

3. Results

The mean (± SD) age of the 517 participants was 38 (± 8) years while their mean (± SD) body mass index (BMI) was 27 (± 6) kg/m². About half of the participants were HIV positive (52%, 270/517). The characteristics of the study participants at baseline are shown in Table 1. Participants returned for follow-up visits at a median (IQR) time of 6.0 (5.8–8.1) months.

3.1. Prevalent hrHPV infection

At baseline, about a quarter of the study participants (24%, 125/517) had hrHPV infections. The distribution of type-specific cervical hrHPV Infections by HIV Status at Baseline is shown in Table 2. For subsequent analyses, we compared 125 women with cervical hrHPV infections to 392 women without cervical hrHPV infections at baseline. DQA1*01:02 was the most prevalent HLA allele in the study population (frequency = 0.39). This allele was present in 60% of the women who were hrHPV negative (frequency = 0.36) and 71% of the women who were hrHPV positive (frequency = 0.46), Table 3. Supplemental Table 1 shows the frequency distribution of all the HLA alleles identified among the study participants at baseline.

Several HLA alleles were associated with prevalent hrHPV infections (Table 4, Fig. 1). In unadjusted regression models (model 1), the odds ratio (OR), 95% confidence interval (CI) and *p* values were 0.35

Table 1
Characteristics of the study participants at baseline by cervical hrHPV Status.

Characteristics	Total (n = 517)	hrHPV Negative (n = 392)	hrHPV Positive (n = 125)
	Mean (± standard deviation)		
Age, years	38 (± 8)	38 (± 8)	37 (± 7)
Body Mass Index, kg/m^2	27 (± 6)	27 (± 8)	27 (± 8)
Age at sexual initiation, years	20 (± 4)	20 (± 4)	19 (± 3)
Total sex partners	3 (± 3)	3 (± 3)	4 (± 3)
	n (%)		
Age, years			
18–29	55 (11)	40 (10)	15 (12)
30– < 45	357 (69)	273 (70)	84 (67)
≥45	105 (20)	79 (20)	26 (21)
Body Mass Index, kg/m^2			
Normal Weight, 18.5–24.9	173 (35)	127 (34)	46 (40)
Overweight, 25.0–29.9	171 (35)	130 (35)	41 (35)
Obese, ≥30.0	145 (30)	116 (31)	29 (25)
Age at sexual initiation, years			
< 18	106 (21)	80 (21)	26 (21)
18–21	248 (49)	188 (49)	60 (49)
≥22	150 (30)	114 (30)	36 (29)
Total sex partners			
1	122 (24)	101 (26)	21 (17)
2–4	202 (40)	152 (39)	50 (41)
≥5	186 (36)	135 (35)	51 (42)
HIV Status			
Positive	270 (52)	182 (46)	88 (70)
Negative	247 (48)	210 (54)	37 (30)

hrHPV = high-risk human papillomavirus.

Table 2
Distribution of type-specific cervical high-risk HPV Infections by HIV Status at Baseline.

HPV Type	Total (n = 517)	HIV Positive (n = 270)	HIV Negative (n = 247)
High-risk HPV Positive	125 (24.2%)	88 (32.6%)	37 (14.9%)
HPV 16	10 (1.9%)	8 (2.9%)	2 (0.8%)
HPV 18	20 (3.9%)	15 (5.5%)	5 (2.0%)
HPV 31	11 (2.1%)	10 (3.7%)	1 (0.4%)
HPV 33	16 (3.1%)	13 (4.8%)	3 (1.2%)
HPV 35	27 (5.2%)	22 (8.1%)	5 (2.0%)
HPV 39	7 (1.3%)	5 (1.8%)	2 (0.8%)
HPV 45	9 (1.7%)	5 (1.8%)	4 (1.6%)
HPV 51	9 (1.7%)	8 (2.9%)	1 (0.4%)
HPV 52	37 (7.1%)	25 (9.2%)	12 (4.8%)
HPV 56	9 (1.7%)	5 (1.8%)	4 (1.6%)
HPV 58	10 (1.9%)	8 (2.9%)	2 (0.8%)
HPV 59	7 (1.3%)	7 (2.5%)	0 (0.0%)

(0.15–0.84), *p* 0.13 for C*07:02, 1.52 (1.13–2.03), *p* 0.03 for DQA1*01:02, 1.78 (1.08–2.92), *p* 0.05 for DQA1*02:01 and 0.49 (0.26–0.91), *p* 0.05 for DQA1*05:01. With adjustment for age and HIV status (model 2), most of these effect sizes became larger. With further adjustment for the first principal component in the fully adjusted models (model 3), DQA1*01:02 (OR: 1.46, 95% CI: 1.08–1.97, *p* 0.03), DQA1*02:01 (OR: 1.89, 95% CI: 1.12–3.15, *p* 0.03) and DQA1*05:01 (OR: 0.45, 95% CI: 0.24–0.85, *p* 0.03) were significantly associated, while C*07:02 (OR: 0.30, 95% CI: 0.13–0.75, *p* 0.06) was borderline significantly associated with cervical hrHPV infection at baseline (Table 4). Results of the zygosity test for these HLA alleles showed that among women with one C*07:02 or DQA1*01:02 allele, heterozygosity for that allele was associated with higher odds of cervical hrHPV infections. While the presence of one homozygous and one heterozygous DQA1*02:01 allele, was associated with higher odds of cervical hrHPV

infections (Table 5). Additionally, four haplotypes, A*30:01 – DQA1*05:01, B*07:02 – C*07:02, B*07:02 – DQA1*05:01 and C*07:02 – DQA1*05:01, were significantly associated with prevalent cervical hrHPV infections at baseline (Table 6).

Next, we examined the association between amino acid positions and cervical hrHPV infections. We found that 6 amino acid positions on DQα1 were significantly associated with prevalent hrHPV infections at baseline. The position_amino acid residues were 230_Methionine, 70_Lysine, 75_Histidine, 77_Leucine, 75_Arginine, 98_Serine (Table 7).

3.2. Persistent hrHPV infection

We compared 51 women with hrHPV infection at both the baseline and follow-up visits to 355 women without hrHPV infections at either the baseline or follow-up visits. DQA1*01:02 was the most common HLA allele among the 406 women (frequency = 0.37). This allele was present in 59% of the women who were persistently cervical hrHPV negative (frequency = 0.36) and 67% of the women who were persistently cervical hrHPV positive (frequency = 0.40) – Fig. 1. The frequency distribution of the top 10 alleles and all the alleles identified are shown in Table 3 and Supplemental Table 2, respectively.

In the unadjusted regression analyses, four alleles were significantly associated with higher odds of persistent cervical hrHPV infections (B*15:10, OR: 2.73, 95% CI: 1.38–5.42, *p* 0.03; DQA1*05:01, OR: 2.56, 95% CI: 1.36–4.82, *p* 0.02; DQB1*02:01, OR: 2.30, 95% CI: 1.21–4.36, *p* 0.04; DRB1*03:01, OR: 2.60, 95% CI: 1.37–4.96, *p* 0.03), while one allele (DQB1*05:01, OR: 0.34, 95% CI: 0.14–0.81, *p* 0.04) was significantly associated with lower odds of persistent cervical hrHPV infections (Table 4). With further adjustments for age, HIV status and the first principal component, all the effect estimates were attenuated and the associations became borderline statistically significant (B*15:10, DQB1*02:01, DQB1*05:01) or not significant (DRB1*03:01), except for DQA1*05:01, which remained relatively the same in all the regression models. In the fully adjusted model, DQA1*05:01 (OR: 1.46, 95% CI: 1.08–1.97, *p* 0.04) was still significantly associated with higher odds of persistent cervical hrHPV infection. The results of the zygosity tests showed that among women with one DQA1*05:01 allele, heterozygosity for that allele was associated with lower odds of persistent cervical hrHPV infections, OR: 0.45, *p* value 0.02 (Table 5). Several haplotypes that included the DQA1*05:01 allele, were also found to be significantly associated with persistent cervical hrHPV infections, including B*15:10 – DQA1*05:01, DQA1*05:01 – DQB1*02:01 and DQA1*05:01 – DRB1*03:01 (Table 6). While we found several amino acids associated with persistent cervical hrHPV infections, none of the significant amino acid residues were located on DQα1 (Table 7). The results of the zygosity tests for the amino acid residue are shown in Supplemental Table 3.

In sensitivity analysis, we observed that DPB1*01*01 was the most prevalent allele (frequency = 0.42) among women with type-specific cervical hrHPV infections (n = 37) and DQA1*05*05 was significantly associated with lower odds of type-specific persistent cervical hrHPV infections (OR: 0.09, 95% CI: 0.01–0.47, *p* 0.01).

4. Discussion

This is the first study to examine the association between HLA alleles, haplotypes and amino acids, and cervical hrHPV infections among African women. Notably, DQA1*01:02 and DQA1*02:01 were positively associated with prevalent cervical hrHPV infections. On the other hand, DQA1*05:01 was associated with 55% lower odds of prevalent cervical hrHPV infections. However, hrHPV infections were more likely to be persistent among women with DQA1*05:01. Several haplotypes that include DQA1*05:01 were associated with persistent hrHPV but none of the amino acid residues located on DQα1 were associated with persistent cervical hrHPV infections in this study population.

HLA- DQA1*01:02 was the most common allele in the study

Table 3
Frequency distribution of top 10 HLA alleles by cervical hrHPV status.

HLA Allele	Total (n = 517)		hrHPV Negative (n = 392)			hrHPV Positive (n = 125)		
	Count	Frequency	Allele Count	Allele Frequency	Population Frequency	Allele Count	Allele Frequency	Population Frequency
Prevalent hrHPV								
DQA1*01:02	402	0.388	286	0.364	0.602	116	0.464	0.712
DPB1*01:01	355	0.343	273	0.348	0.553	82	0.328	0.560
DQB1*06:02	284	0.274	204	0.260	0.443	80	0.320	0.520
C*04:01	282	0.272	210	0.267	0.451	72	0.288	0.472
DRB1*15:03	187	0.180	134	0.170	0.298	53	0.212	0.360
DPB1*02:01	181	0.175	133	0.169	0.301	48	0.192	0.360
B*53:01	180	0.174	133	0.169	0.306	47	0.188	0.320
DQB1*03:01	174	0.168	138	0.176	0.324	36	0.144	0.248
A*23:01	168	0.162	125	0.159	0.290	43	0.172	0.336
DQA1*05:05	146	0.141	113	0.144	0.262	33	0.132	0.208
Persistent hrHPV								

HLA Allele	Total (n = 406)		Persistent hrHPV Negative (n = 355)			Persistent hrHPV Positive (n = 51)		
	Count	Frequency	Allele Count	Allele Frequency	Population Frequency	Allele Count	Allele Frequency	Population Frequency
DQA1*01:02	298	0.367	257	0.362	0.594	41	0.402	0.666
DPB1*01:01	276	0.339	234	0.3296	0.521	42	0.411	0.666
C*04:01	216	0.266	192	0.2704	0.459	24	0.235	0.333
DQB1*06:02	214	0.263	187	0.2634	0.445	27	0.264	0.431
DRB1*15:03	147	0.181	131	0.1845	0.318	16	0.156	0.254
DQB1*03:01	142	0.174	129	0.1817	0.329	13	0.127	0.254
B*53:01	140	0.172	124	0.1746	0.312	16	0.156	0.294
DPB1*02:01	137	0.168	122	0.1718	0.307	15	0.147	0.274
A*23:01	134	0.165	112	0.1577	0.290	22	0.215	0.392
DQA1*05:05	122	0.150	112	0.1577	0.281	10	0.098	0.196

hrHPV = high-risk human papillomavirus.

population (allele frequency 39%, population frequency 63%). Although DQA1*01:02 has not been previously reported in Nigeria, it's frequency is high in other populations in SSA, including Gabon (allele frequency 50%), Congo (allele frequency 42%, population frequency 53%), Cameron (allele frequency 38%) and Kenya (allele frequency 32%, population frequency 57%), as well as in African Americans in the United States (allele frequency 31%, population frequency 48%) [31]. DQA1*01:02 was associated with higher odds of prevalent cervical hrHPV infection in the present study. This HLA allele was also associated with cervical intraepithelial neoplasia (CIN) in American Indian women, OR 4.5, 95% CI 1.3–5.3 [32]. It has also been linked to other viral diseases including JC polyomavirus and Merkel cell polyomavirus [33], immune-mediated disorders including type 1 diabetes [34], systematic lupus erythematous (SLE) [35], multiple sclerosis and celiac disease [36], as well as other disorders including tuberculosis [35] and narcolepsy [36,37]. DQA1*02:01 was also associated with higher odds of prevalent cervical hrHPV infection in this study. This variant has been linked to other cancers including nasopharyngeal carcinoma [38] and chronic myelogenous leukemia [39]. It was also associated with seropositivity of HPV 77 [40], an α cutaneous HPV genotype linked to common warts and skin cancer [41–43]. Therefore, these two HLA alleles seem to be pleiotropic for several virus infections, virus-associated cancers and autoimmune disorders.

The frequency of DQA1*05:01 was lower in our population (allele frequency 8%), than in other populations, with frequencies of 60% reported from Argentina, 78% in Colombia, and 89% in the United States [31]. We were unable to compare our finding that DQA1*05:01 and several haplotypes that include this variant were associated with hrHPV persistence to results from other studies, given the dearth of data. Nonetheless, the association between DQA1*05:01 and cervical neoplasia has been examined in some studies and the results have been inconsistent. A study among Honduran women reported that the frequency of DQA1*05:01 was lower among women with CIN III or cervical cancer (n = 5), compared to those with CIN I or II (n = 12), RR: 0.30, *p* 0.03 [44]. This allele was more common among Norwegian

women with CIN II-III compared to those without CIN (OR: 2.4, 95% CI: 1.0–5.4 *p* 0.02) [45]. While one study found DQA1*05:01-DQB1*0301 haplotype was negatively associated with cervical dysplasia and neoplasia in HPV16-positive women (OR: 0.19, 95% CI: 0.06–0.50 *p* < 0.001) [46], another study showed that this haplotype was positively associated with cervical neoplasia among HPV16-seronegative women (OR: 3.00 *p* < 0.01) [47]. These results may be due to non-16 hrHPV types that were not examined in the previous studies, such as HPV 31, 33, 35, 45, 52 and 58, which account for 20% of cervical cancers worldwide. These non-16 hrHPV are more common than HPV16 in our study population [2,4,20,21,48] and may explain the association between C and hrHPV persistence observed in this study. This variant and other haplotypes that include it have also be linked polycystic ovary syndrome [49], ovarian cancer [50] and autoimmune diseases including type 1 diabetes and celiac disease [36]. DQA1*05:05, which was associated with significantly lower odds of type-specific persistent hrHPV infections in our study, was also found to be associated with reduced odds in female patients with early onset breast cancer, compared to healthy controls [51].

Although we observed an association between DQB1*02:01 and persistent cervical hrHPV infections, the association was borderline significant after adjustment for age, HIV status and the first principal component (OR: 2.10, 95% CI: 1.08–4.09, *p* 0.08). Alifu et al. reported that the frequency of DQB1*02:01 was higher among Uyghur women with advanced squamous cell cervical cancer compared to otherwise healthy controls in China [8]. The small size of our study may be responsible for attenuation of the *p* values we observed. Conversely, we observed DQB1*05:01 was associated with 66% lower odds of persistent hrHPV infection and this association was also attenuated to become borderline significant in the fully adjusted models (OR: 0.34, 95% CI: 0.14–0.82, *p* 0.08). In support of this finding, DQB1*05:01 allelic variant has been shown to be protective of cervical cancer in different populations. Brazilian [18] and British [52] women with DQB1*05:01 and Chinese women with DQB1*05:02:01 [53], were less likely to have cervical cancer compared to otherwise healthy women.

Table 4
Association between HLA alleles and cervical hrHPV infection.

Models	Prevalent hrHPV					Persistent hrHPV				
	Allele	Frequency Controls	Frequency Cases	Odds Ratio (95% CI)	P value* (Adjusted)	Allele	Frequency Controls	Frequency Cases	Odds Ratio (95% CI)	P value* (Adjusted)
Model 1				1.00 (Ref)					1.00 (Ref)	
	B*07:02	0.077	0.036	0.429 (0.207–0.887)	0.157	B*15:10	0.042	0.117	2.736 (1.381–5.421)	0.027
	C*07:02	0.063	0.024	0.353 (0.149–0.842)	0.132	DQA1*05:01	0.078	0.166	2.560 (1.359–4.819)	0.021
	DQA1*01:02	0.364	0.464	1.518 (1.132–2.035)	0.031	DQB1*02:01	0.080	0.156	2.304 (1.215–4.361)	0.044
	DQA1*02:01	0.059	0.104	1.779 (1.084–2.920)	0.049	DQB1*05:01	0.146	0.058	0.337 (0.141–0.808)	0.044
	DQA1*05:01	0.096	0.052	0.489 (0.262–0.912)	0.049	DRB1*03:01	0.071	0.156	2.604 (1.366–4.962)	0.032
Model 2				1.00 (Ref)					1.00 (Ref)	
	B*07:02	0.077	0.036	0.400 (0.191–0.839)	0.108	B*15:10	0.042	0.117	2.585 (1.259–5.307)	0.067
	C*07:02	0.063	0.024	0.304 (0.126–0.737)	0.058	DQA1*05:01	0.078	0.166	2.470 (1.277–4.777)	0.043
	DQA1*01:02	0.364	0.464	1.454 (1.076–1.966)	0.030	DQB1*02:01	0.080	0.156	2.099 (1.078–4.083)	0.087
	DQA1*02:01	0.059	0.104	1.900 (1.136–3.177)	0.030	DQB1*05:01	0.146	0.058	0.336 (0.138–0.881)	0.087
	DQA1*05:01	0.096	0.052	0.456 (0.242–0.859)	0.030	DRB1*03:01	0.071	0.156	2.375 (1.212–4.657)	0.105
Model 3				1.00 (Ref)					1.00 (Ref)	
	B*07:02	0.077	0.036	0.394 (0.188–0.828)	0.099	B*15:10	0.042	0.117	2.602 (1.264–5.356)	0.065
	C*07:02	0.063	0.024	0.309 (0.128–0.749)	0.065	DQA1*05:01	0.078	0.166	2.465 (1.274–4.768)	0.043
	DQA1*01:02	0.364	0.464	1.457 (1.076–1.972)	0.033	DQB1*02:01	0.080	0.156	2.103 (1.080–4.093)	0.085
	DQA1*02:01	0.059	0.104	1.880 (1.122–3.151)	0.033	DQB1*05:01	0.146	0.058	0.339 (0.140–0.823)	0.085
	DQA1*05:01	0.096	0.052	0.449 (0.238–0.847)	0.033	DRB1*03:01	0.071	0.156	2.376 (1.212–4.657)	0.105

hrHPV = high-risk human papillomavirus.
Reference Category = hrHPV negative.
Model 1 = Unadjusted model.
Model 2 = Adjusted for age and HIV status.
Model 3 = Adjusted for age, HIV status and the first principal component.
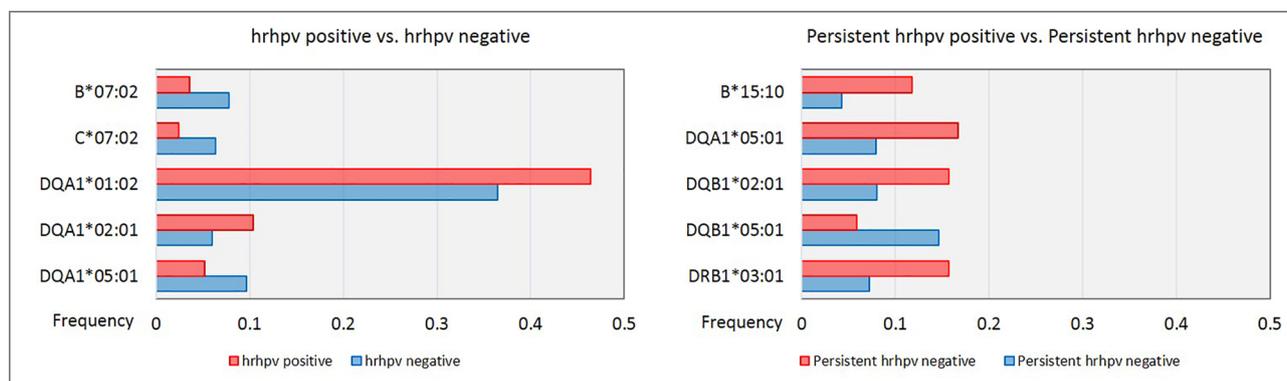* = FDR-adjusted P value.

Fig. 1. Frequency distribution of alleles associated with prevalent and persistent cervical hrHPV.

Table 5
Zygosity tests for HLA alleles associated with cervical hrHPV.

Allele	Homozygosity		Heterozygosity		Zygosity	
	OR	P value	OR	P value	OR	P value
Prevalent hrHPV						
B*07:02	0.492	0.999	1.876	0.079	0.262	0.336
C*07:02	2.087	0.999	2.194	0.022	0.951	0.999
DQA1*01:02	2.487	0.999	2.595	0.018	0.958	0.999
DQA1*02:01	1.625	0.107	0.695	0.133	2.334	0.006
DQA1*05:01	1.933	0.606	0.564	0.061	3.423	0.224
Persistent hrHPV						
B*15:10	5.588	0.155	0.426	0.062	13.11	0.036
DQA1*05:01	10.93	0.222	0.432	0.017	25.30	0.107
DQB1*02:01	11.89	0.208	0.484	0.046	24.53	0.110
DQB1*05:01	2.164	0.999	2.709	0.015	0.799	0.999
DRB1*03:01	10.65	0.227	0.425	0.022	25.02	0.108

hrHPV = high-risk human papillomavirus; ID = HLA Gene_Position_Amino acid residue; A = Alanine; D = Aspartic acid; E = Glutamic acid; H = Histidine; I = Isoleucine; K = Lysine; L = Leucine; M = Methionine; R = Arginine; S = Serine.

The results of our present study provide evidence for the role of specific HLA alleles in determining susceptibility to persistent cervical hrHPV infections, a precursor for cervical cancer. To promote immune recognition and subsequent clearance of infected cells, Class I HLA molecules present foreign antigens, such as HPV peptides to CD8 cytotoxic T lymphocytes, while class II HLA molecules present antigenic peptides to CD4 T helper cells [54]. HLA molecules that bind HPV-specific antigens facilitate viral clearance, while HLA molecules that fail to recognize and present HPV-specific antigens may promote viral persistence. Cytotoxic T lymphocytes responses to HPV infection have been well documented [55,56]. Downregulation and loss of HLA class I antigen expression enable HPV-infected cells to escape detection by the immune system by becoming nonimmunogenic and this has been reported in cervical cancer [57,58]. Our results suggest that DQA1*05:01 may be associated with immune evasion of host defense and promotion of hrHPV persistence, thereby facilitating development of cervical malignancy.

Our study has several important limitations. First, even though our sample size was bigger than that in some of the previous studies, we may have missed associations with smaller effect sizes, and we had inadequate power to examine the relationship between HLA alleles and type-specific hrHPV. However, examining the associations between HLA alleles and group hrHPV identified significant results and confirmed findings in other populations. Second, our study was limited by the short duration of follow up of 6–8 months. While a longer follow up of up to 12 months may have be useful, a meta-analysis of 86 previous studies which provided data on over 100,000 women globally, showed that a follow-up period of at least 6 months was sufficient to define persistent hrHPV infections [59]. Lastly, although we did not have HIV clinical information such as duration of HIV infection, use of anti-retroviral therapy, viral load or CD4 count that may be associated with clearance of HPV infection among HIV-positive women, we excluded HIV-positive women who were severely ill from our analyses. However, in another study where clinical features of HIV were examined in relation to HPV, no association was found [60]. The strengths of our study include studying a well-characterized longitudinal cohort, collecting detailed data on numerous exposures using validated procedures and conducting multiple hrHPV assessments in the participants.

In summary, we report the frequency distribution of HLA alleles and the association between the alleles and haplotypes and their association with cervical hrHPV infections in Nigerian women. We found some HLA allelic variants, haplotypes and amino acid positions were significantly associated with prevalent and persistent cervical hrHPV infections. Future large-scale studies that examine the association between HLA allelic variants and persistence of type-specific hrHPV infections and cervical cancer would be informative. These studies may contribute to a better understanding of the role of HLA allelic variants in individualized medicine and clinical decision making and the relationship between HPV infections and the host immune response that underpin the development of persistent infection and cervical carcinogenesis.

Table 6
HLA Haplotypes Associated with Cervical hrHPV Infections.

Prevalent hrHPV			Persistent hrHPV		
Allele	OR	P value	Allele	OR	P value
A*30:01 – DQA1*05:01	0.99	0.025	B*15:10 – DQA1*05:01	4.53	0.005
B*07:02 – C*07:02	0.33	0.009	B*15:10 – DQB1*02:01	4.38	0.006
B*07:02 – DQA1*05:01	0.09	0.025	B*15:10 – DRB1*03:01	4.47	0.005
C*07:02 – DQA1*05:01	0.10	0.024	DQA1*05:01 – DQB1*02:01	2.49	0.013
			DQA1*05:01 – DRB1*03:01	2.54	0.012
			DQB1*02:01 – DRB1*03:01	2.46	0.013

hrHPV = high-risk human papillomavirus.

Table 7

Amino Acids Associated with Cervical hrHPV Infections.

Prevalent hrHPV

ID	Number of Controls		Number of Cases		Odds Ratio (OR)	P value (Unadjusted)	P valueAdjusted
	Carriers	Non-Carriers	Carriers	Non-Carriers			
C_123_S	49	343	6	119	0.38	0.012	0.012
DQA1_230_M	236	156	89	36	1.62	0.033	0.033
DQA1_70_K	45	347	24	101	1.84	0.034	0.034
DQA1_75_H	45	347	24	101	1.84	0.034	0.034
DQA1_77_L	45	347	24	101	1.84	0.034	0.034
DQA1_75_R	253	139	63	62	0.56	0.006	0.042
DQA1_98_S	170	222	37	88	0.55	0.006	0.012

Persistent hrHPV

ID	Number of Controls		Number of Cases		Odds Ratio (OR)	P value (Unadjusted)	P value Adjusted
	Carriers	Non-Carriers	ID	Carriers			
DQB1_98_D	130	225	31	20	2.66	0.001	0.003
DQB1_99_I	130	225	31	20	2.66	0.001	0.003
DQB1_89_A	107	248	26	25	2.4	0.004	0.012
DQB1_103_K	101	254	24	27	2.23	0.009	0.018
DQB1_106_A	101	254	24	27	2.23	0.009	0.018
DQB1_60_S	101	254	24	27	2.23	0.009	0.018
DQB1_62_S	101	254	24	27	2.23	0.009	0.018
DQB1_69_I	101	254	24	27	2.23	0.009	0.018
DQB1_78_E	101	254	24	27	2.23	0.009	0.018
DQB1_84_L	101	254	24	27	2.23	0.009	0.009
DQB1_87_L	101	254	24	27	2.23	0.009	0.009
DQB1_62_H	150	205	10	41	0.35	0.001	0.009
DQB1_103_A	125	230	8	43	0.36	0.006	0.012
DQB1_46_L	125	230	8	43	0.36	0.006	0.012

hrHPV = high-risk human papillomavirus; ID = HLA Gene_Position_Amino acid residue; A = Alanine; D = Aspartic acid; E = Glutamic acid; H = Histidine; I = Isoleucine; K = Lysine; L = Leucine; M = Methionine; R = Arginine; S = Serine.

Conflict of interest

The authors have no conflict of interest to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humimm.2019.04.011.

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