HLA Class II DR-DQ and Increased Risk of Cervical Cancer among Senegalese Women[1]

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Abstract

To examine Senegalese women to confirm and extend associations between HLA class II types and cervical cancer previously observed among African-American, Caucasian, Hispanic, and Japanese ethnic populations, 55 Senegalese women with invasive cervical carcinoma were compared with age-matched (human papillomavirus) HPV-positive ($n = 83$) and HPV-negative ($n = 107$) control women. PCR-based HPV and HLA typing methods were used. Data were analyzed using a global randomization test and conditional logistic regression. Although this study failed to confirm a previously reported association between cervical cancer and *DQB1*03* alleles, the *DRB1*1101-DQB1*0301* haplotype was detected more frequently among cervical carcinoma cases than among controls (adjusted odds ratio, 2.6; 95% confidence interval, 1.0–7.1). Furthermore, as reported by others, we observed a negative association of borderline statistical significance between *DRB1*13* and cervical carcinoma (adjusted odds ratio, 0.5; 95% confidence interval, 0.2–1.1). Observations from this study confirm earlier findings of a negative association between *DRB1*13* and cervical cancer and suggest that specific *DRB1-DQB1* haplotype combinations, rather than individual *DQB1*03* alleles, increase the risk for cervical cancer.

Introduction

Strong epidemiological evidence suggests that persistent infection with specific "high-risk" types of genital HPV[3] leads to development of cervical cancer (1, 2), the third most frequent cancer in women worldwide, with approximately 371,200 new cases reported every year (3). HPV DNA has been found in nearly 100% of cervical cancers (4, 5), but in only 17–44% of control tissues (6). However, current evidence suggests that most HPV infections are self-limited and that only a minority of untreated cervical HPV infections progress to cervical cancer (7). Why only some untreated women infected with a high-risk HPV type develop invasive cancer is unclear; however one potential cofactor may be the host's cellular immune response to HPV infection.

HLAs are cell surface proteins that play an important role in human cell-mediated immunity, with HLA class I proteins present on all cells and HLA class II proteins present on antigen-presenting cells. Several HLA types have been found to be associated with an increased risk of various diseases. Previous studies among African-American, Caucasian, Hispanic, and Japanese ethnic populations report associations between cervical neoplasia and *DRB1*1101-DQB1*0301* haplotype (8, 9), *DQB1*03* alleles (8, 10–22), *DR5* (*DRB1*11*, *DRB1*12*) alleles (21, 23, 24), *DQB1*0201* (14, 17), and *DQB1*06* (13, 15, 17, 19, 25). However, disparate findings have been reported in different populations, possibly because of differences in HPV infection status or the extent to which preneoplastic lesions were discovered and treated before cancer developed. The present case-control study examined associations between invasive cervical carcinoma and specific HLA alleles *DQB1*03*, *DRB1*11*, *DQB1*0201*, *DQB1*06* and HLA haplotype *DRB1*1101-DQB1*0301* among a population of Senegalese women with and without HPV infection who had never undergone cervical cytological screening or treatment of preneoplastic lesions. Thus, our ability to distinguish associations of HLA alleles with invasive cervical cancer from those between HLA alleles and HPV infection (often manifested by morphological changes consistent with low-grade squamous intraepithelial lesions which frequently regress), in addition to the vast allelic diversity found among African and African-derived populations (26), provides a unique context for the interpretation of the role of specific HLA alleles in the etiology of cervical cancer.

Materials and Methods

Study Population. Between March and December of 1998, all women 35 years of age or older presenting to the University of Dakar (Senegal) Oncology Clinic, the main cancer referral clinic in Senegal, with signs or symptoms of invasive cervical cancer were evaluated by colposcopy and biopsy to confirm the presence of invasive cervical cancer. A total of 55 women with histologically confirmed invasive disease were enrolled. Two control groups, individually matched to cases by age (±3 years), were recruited from among 1201 women 35 years of age or older seeking primary care or who presented for family planning purposes to the Pikine Outpatient Clinic of the University of Dakar during the same time period. All cases and potential controls provided written informed consent according to procedures approved by the institutional review boards at the

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[3] The abbreviations used are: HPV, human papillomavirus; OR, odds ratio; CI, confidence interval; CIN, cervical intraepithelial neoplasia.

University of Dakar and the University of Washington. The HPV-positive control group consisted of 83 women with normal cervical cytology findings and positive HPV DNA cervical swab results. The HPV-negative control group included 107 women with normal cytology findings and negative HPV DNA results. Controls were matched to the cases by age because HPV DNA detection is highly associated with age (27–32) and cancer incidence. Although we attempted to match each cervical carcinoma case with two controls, only 83 HPV-positive controls could be matched to the 55 cervical cancer cases because the referral population included too few cytologically normal women with positive tests for HPV DNA. All cases were matched to at least one control, but five cases (68–83 years of age) could not be matched with an HPV-positive control. At the time of the enrollment examination, all cases and controls had an intact cervix and were not pregnant.

Study Procedures and Collection of Specimens. All subjects completed a short standardized interview, including questions concerning medical, gynecological, and sexual history, and underwent a physical and gynecological examination. Cervical cellular samples were obtained for cytological screening, detection of HPV DNA and for analysis of HLA class II loci (*DRB1* and *DQB1*). Colposcopically directed biopsy specimens of the uterine cervix were obtained from all women with suspected cancer.

Detection and Typing of HPV. Cervical swab specimens were tested for presence and type of HPV using a PCR-based assay with L1 consensus primers MY09 and MY011 and then hybridization with a biotin-labeled generic HPV probe. If the sample tested positive using the generic probe, it was amplified again with biotinylated primers (MY09/MY11/HMB01). HPV detection was then performed through probe hybridization using a reverse-line blot-strip detection method, as described by Gravitt *et al.* (33). The HPV strip method signaled the presence of HPV DNA with two control beta-globin probes and typed for 27 specific genital HPV types.

HLA Typing. Human DNA was extracted from cervical swab samples and prepared for PCR amplification. All samples were typed for two HLA class II loci, *DQB1* and *DRB1*. Primers were provided by Roche Molecular Systems (Alameda, California). HLA class II line-blot typing strips and all liquid detection reagents were from the Amplicor Strip Detection Reagent Kit manufactured by Dynal (Oslo, Norway). HLA class II typing used single-stranded oligonucleotide probe reverse-dot blot methods as described by Erlich *et al.* (34) and Saiki *et al.* (35).

DQB1 was amplified using 2–5 μl of sample with a single amplification using biotinylated primers DB378B/DB380B. Forty PCR cycles were conducted on a Perkin-Elmer GeneAmp PCR System 9600 using the following cycling conditions: 95°C denaturation for 15 s; 60°C annealing for 45 s; and 72°C extension for 15 s. Amplified DNA was denatured and hybridized onto DQB1 reverse line-blot typing strips in 20-well typing trays immersed in 4× saline-sodium phosphate-EDTA-0.1% SDS hybridization and 1× saline-sodium phosphate-EDTA-0.01% SDS stringent wash solutions in a 50°C shaking water bath. Color detection was performed using streptavidin-horseradish peroxidase color conjugate and a hydrogen peroxidase-tetramethylbenzidine mixture. Positive probe patterns of the 25 immobilized sequence-specific oligonucleotide probes were entered into a computerized *DQB1* pattern-matching program, which were interpreted or deciphered into *DQB1*-specific alleles. This typing method is able to detect and differentiate between 30 different *DQB1* alleles.

General *DRB* typing was amplified using biotinylated primers RC1000/CRX28B with the same PCR cycling conditions described above for the *DQB1* locus. Amplified PCR product was denatured and hybridized onto DRB general strips with 29 sequence-specific immobilized probes using the same hybridization procedures and solutions described for the *DQB1* locus. The general typing strips categorized samples into DRB1 sero-groups, *DRB1*-specific allele possibilities, and some DRB3, DRB4, and DRB5 types, which were not used in this analysis. Because of the complexity of the *DRB1* locus, subgroup amplification was necessary for specific *DRB1* allele differentiation. The DRB1 general amplification defined *DRB1*07, DRB1*09,* and *DRB1*10* alleles, which did not need to be specified further. All other samples underwent a second round of amplification using group-specific biotinylated primers. The DR1 group was amplified with primers DB1151/DB1035. The DR2 group was amplified using primers DB1150/DB1035. The DR3 group was amplified using primers DB1146/DB1035. The DR4 group was amplified with DB1034/DB1035. The DR8 group was amplified using primers DB1148/DB1035. Group-specific amplification methods and procedures were typed using the same hybridization protocol as described for the *DQB1* and *DRB1* general loci, but used 30-probe DRB1-specific strips. Positive probe patterns were entered into a computerized pattern-matching program or matched using a probe-pattern-typing grid to determine specific *DRB1* allele type. This DRB typing method was able to differentiate between 131 different *DRB1* alleles.

Haplotype configuration was determined according to known linkage disequilibrium patterns (36). HLA typing was performed by one laboratory technician who was unaware of the subject's case-control and HPV status.

Histopathological and Cytological Methods. Cervical cytological samples, collected directly into PreservCyt (Cytyc Corporation, Boxborough, MA) for liquid-based cytology (ThinPrep 2000; Cytyc Corporation), were stained and evaluated as described previously (37) without knowledge of other clinical or laboratory data. Representative H&E-stained slides were prepared from paraffin-embedded biopsies, and reviewed by the pathologist (N. K.) without knowledge of other clinical or laboratory data. Standard gynecological pathology criteria and terminology were used to classify all intraepithelial lesions and invasive cervical cancers (38).

Statistical Analysis. To reduce the possibility of reporting spurious associations which might have been detected solely because of the large number of comparisons needed to assess all of the individual HLA types, a two-step approach was taken in the analysis of these data. First, associations between cervical cancer and particular HLA types that had been reported previously more than once among other ethnic populations were assessed in our population by conditional logistic regression, which took into account the individual matching by age. Second, a global randomization test was used to determine whether any of the remaining HLA types (that is, those types not hypothesized *a priori* to be associated with cervical cancer) were associated with cervical cancer beyond what might be expected to occur by chance alone.

Because controls were individually matched to the cases by age, conditional logistic regression analyses were used to obtain ORs, 95% CIs, and *P*s assessing *a priori* hypothesized associations between *HLA* alleles/haplotypes and invasive cervical cancer. Confounding of the association between cervical carcinoma and certain HLA alleles was ascertained by identifying characteristics that varied in distribution between cases

and controls. A factor was considered to be a potential confounder if the characteristic also differed substantially with the presence or absence of HLA alleles among controls and changed the crude OR by >10%. Analyses were performed comparing cases to HPV-positive and HPV-negative control groups separately. The control groups were combined for the final analyses because there were no significant differences between the two groups. Statistical analysis was performed using SAS v.6.12 (Cary, NC).

The association between HLA type and case-control status was also assessed by means of a randomization test. First, the observed χ^2 statistic comparing cases with each control group was calculated. The case-control indicator was then randomly permuted within each matched stratum and the χ^2 statistic was recomputed. This procedure was repeated 1000 times, providing a distribution of the χ^2 statistic under the null hypothesis of no association between case-control status and HLA haplotype. A P was calculated by comparing the observed χ^2 statistic with this null distribution.

We also compared the risk of cervical cancer associated with HLA homozygosity (for *DRB1* and *DQB1* alleles) with heterozygosity using conditional logistic regression analyses, because homozygosity may confer a greater cancer risk inasmuch as less variation in the HLA region may be associated with a narrower range of specific antigens recognized by the host immune system.

Results

Characteristics of the Study Population. The mean age of women enrolled was 48 years (range, 35–83 years). Although the controls were age-matched (±3 years) to the cases, HPV-positive controls were significantly younger than cases (45.8 years *versus* 49.5 years; $P < 0.05$; Table 1). Women in both control groups were similar to cases with respect to ethnicity, marital status (including polygamous *versus* monogamous marriage), and smoking habits. A higher proportion of cases, as compared with HPV-positive controls and HPV-negative controls, were born outside of Dakar ($P < 0.05$).

Women with cervical carcinoma tended to have fewer children (mean, 4.9) than did women in the control groups (HPV-positive, mean, 5.8; HPV-negative, mean, 6.1). Women in both control groups were more likely than cancer cases to practice contraception; however, no one reported the use of condoms.

Detection of HPV. Of the women with cervical carcinoma, 64.7% tested positive for HPV DNA, with 37.3% positive for HPV 16, 7.8% for HPV 18, and 18.2% for other HPV types. Of the HPV-positive controls, 4.8% tested positive for HPV type 16, 10.8% tested positive for HPV type 18, and 84.3% were positive for other HPV types.

Distribution of HLA Haplotypes among Cases and Controls. Class II HLA distributions for the DQB1 and DRB1 were compared among HPV-positive controls, HPV-negative controls, and cervical cancer cases (Table 2). Of the possible *131 DRB1* and *30 DQB1* alleles that could be ascertained with the assay used in this study, 25 different *DRB1* alleles and 13 *DQB1* alleles were actually detected.

Hypothesized HLA Associations between Cervical Cancer Cases and HPV-positive and HPV-negative Controls. Comparison of cervical carcinoma cases to the HPV-positive controls with respect to the class II HLA alleles and haplotypes that were hypothesized *a priori* to be associated with cervical cancer revealed no statistically significant associations (Table 3).

Table 1 Demographic characteristics of cervical carcinoma cases and HPV-positive and HPV-negative controls

Characteristic	Cases $n = 55$	Controls	
		HPV-positive $n = 83$	HPV-negative $n = 107$
Age (mean yr ± SD)	49.5 ± 12	45.8 ± 8[a]	47.9 ± 10
Ethnicity			
Wolof	22 (40.0%)	21 (51.2%)[b]	26 (54.2%)[b]
Pular	15 (27.3%)	8 (19.5%)	11 (22.9%)
Other	18 (32.7%)	12 (29.1%)	11 (23.3%)
Origin (birthplace)			
Dakar	35 (63.6%)	83 (100.0%)[a]	104 (97.2%)[a]
Other	20 (36.7%)	0 (0%)	3 (2.8%)
Marital status			
Monogamously married	13 (23.6%)	22 (26.5%)	29 (27.4%)[c]
Polygamously married	26 (47.3%)	43 (51.8%)	58 (54.7%)
Unmarried	16 (29.1%)	18 (21.6%)	19 (17.9%)
Mean no. of live-born infants ± SD	4.9 ± 3	5.8 ± 2[a]	6.1 ± 3[a]
Current method of birth control			
None	50 (90.9%)	51 (61.4%)[a]	83 (79.0%)[a,d]
Pill	2 (3.6%)	5 (6.0%)	3 (2.9%)
Injection/Norplant	3 (5.5%)	5 (6.0%)	6 (5.7%)
Spermicide	0 (0%)	1 (1.2%)	1 (1.0%)
IUD	0 (0%)	15 (18.1%)	10 (9.5%)
Ligature	0 (0%)	3 (3.6%)	1 (1.0%)
Other	0 (0%)	3 (3.6%)	1 (1.0%)

[a] $P < 0.05$ comparing controls with cases; for birth control method, nonusers were compared with users.
[b] Forty or more missing values.
[c] One missing value.
[d] Two missing values.

A comparison of the frequency of these *DQB1* and *DRB1* alleles between cases and HPV-negative controls revealed a significant association for *DQB1*02* (specifically *DQB1*0201/2*). The *DRB1*1101-DQB1*0301* haplotype was found to be associated with cervical carcinoma, particularly when compared with HPV-negative controls (adjusted OR, 7.4; 95% CI, 1.5–36.5). Although associations between cervical cancer and specific class II HLA alleles and haplotypes did not achieve statistical significance in analyses using the HPV-positive control group, ORs from these analyses were similar in magnitude to those obtained using the HPV-negative control group, suggesting that the control groups could be combined (Table 3). Although the positive association between the *DRB1*1101-DQB1*0301* haplotype and cervical carcinoma was stronger when comparing cases with HPV-negative controls, the association persisted when cases were compared with the combined control groups (adjusted OR, 2.6; 95% CI, 1.0–7.1). The individual *DRB1*1101* and *DQB1*0301* alleles alone were not associated with cervical carcinoma.

Other Potential Allele and Haplotype Associations Examined by Use of a Global Randomization Test. The global randomization test was used to test whether HLA alleles or haplotypes not considered in our *a priori* hypotheses could be associated with invasive cervical cancer in this population. Using this test, no statistically significant difference in *HLA* allele or haplotype distribution between cases and HPV-positive controls ($P = 0.72$) or cases and HPV-negative controls ($P = 0.14$) was observed.

HLA Homozygosity and Cervical Cancer Risk. Women homozygous compared with those heterozygous for an associated *HLA* allele may be at greater risk for developing cervical disease, perhaps caused by less variation in the HLA region. In our study population, 18 (7.4%) had homozygous *DRB1-DQB1*

Table 2 Distribution of class II HLA alleles among cervical carcinoma cases and HPV-positive and HPV-negative controls [*n* (%)]

Allele	Cases ($n = 55$)[a]	Controls		DRB1-DQB1 haplotype	Cases ($n = 55$)[a]	Controls	
		HPV + ($n = 83$)[a]	HPV − ($n = 107$)[a]			HPV + ($n = 83$)[a]	HPV − ($n = 107$)[a]
DRB1							
0101	1 (0.9%)	0 (0.0%)	2 (0.9%)	0101-0501	1 (0.9%)	0 (0.0%)	2 (0.9%)
0102	3 (2.7%)	5 (3.0%)	4 (1.9%)	0102-0501	3 (2.7%)	5 (3.0%)	4 (1.9%)
0301	6 (5.4%)	10 (6.0%)	8 (3.8%)	0301-0201/2	6 (5.5%)	9 (5.4%)	8 (3.7%)
0302	5 (4.5%)	13 (7.8%)	16 (7.5%)	0301-0203	0 (0.0%)	1 (0.6%)	0 (0.0%)
0401	0 (0.0%)	1 (0.6%)	1 (0.5%)	0301-0501	0 (0.0%)	1 (0.6%)	0 (0.0%)
0403	0 (0.0%)	1 (0.6%)	2 (0.9%)	0302-0402	5 (4.5%)	13 (7.8%)	15 (7.0%)
0405	2 (1.8%)	5 (3.0%)	9 (4.2%)	0302-0501	0 (0.0%)	0 (0.0%)	1 (0.5%)
0701	10 (9.1%)	8 (4.8%)	5 (2.4%)	0401-0301	0 (0.0%)	1 (0.6%)	0 (0.0%)
0804	5 (4.5%)	7 (4.2%)	8 (3.8%)	0401-0302	0 (0.0%)	0 (0.0%)	1 (0.5%)
0806	2 (1.8%)	3 (1.8%)	1 (0.5%)	0403-0302	0 (0.0%)	1 (0.6%)	1 (0.5%)
0901	10 (9.1%)	6 (3.6%)	8 (3.8%)	0405-0201/2	2 (1.8%)	1 (0.6%)	2 (0.9%)
1001	17 (15.5%)	17 (10.2%)	28 (13.2%)	0405-0302	0 (0.0%)	4 (2.4%)	0 (0.0%)
1101	12 (10.9%)	22 (13.3%)	13 (6.1%)	0701-0201/2	10 (9.1%)	8 (4.8%)	4 (1.9%)
1102	5 (4.5%)	6 (3.6%)	24 (11.3%)	0701-0203	0 (0.0%)	0 (0.0%)	1 (0.5%)
1104	1 (0.9%)	1 (0.6%)	0 (0.0%)	0804-0301	5 (4.5%)	7 (4.2%)	7 (3.3%)
1201	0 (0.0%)	0 (0.0%)	3 (1.4%)	0804-0604	0 (0.0%)	0 (0.0%)	1 (0.5%)
1202	0 (0.0%)	0 (0.0%)	1 (0.5%)	0806-0602	2 (1.8%)	3 (1.8%)	1 (0.5%)
1301	3 (2.7%)	6 (3.6%)	17 (8.0%)	0901-0201	9 (8.2%)	6 (3.6%)	7 (3.3%)
1302	5 (4.5%)	16 (9.6%)	19 (9.0%)	0901-0203	1 (0.9%)	0 (0.0%)	1 (0.5%)
1303	4 (3.6%)	7 (4.2%)	4 (1.9%)	1001-0501	17 (15.5%)	17 (10.2%)	28 (13.1%)
1304	11 (10.0%)	26 (15.7%)	30 (14.2%)	1101-0203	0 (0.0%)	0 (0.0%)	1 (0.5%)
1322	1 (0.9%)	3 (1.8%)	3 (1.4%)	1101-0301	11 (10.0%)	12 (7.2%)	6 (2.8%)
1401	2 (1.8%)	0 (0.0%)	1 (0.5%)	1101-0501	1 (0.9%)	3 (1.8%)	5 (2.3%)
1503	3 (2.7%)	0 (0.0%)	1 (0.5%)	1101-0502	0 (0.0%)	1 (0.6%)	0 (0.0%)
1602	2 (1.8%)	3 (1.8%)	4 (1.9%)	1101-0602	0 (0.0%)	5 (3.0%)	0 (0.0%)
DQB1				1101-0604	0 (0.0%)	1 (0.6%)	1 (0.5%)
0201/2	27 (24.5%)	26 (15.7%)	24 (11.2%)	1102-0301	5 (4.5%)	5 (3.0%)	23 (10.7%)
0203	1 (0.9%)	1 (0.6%)	3 (1.4%)	1102-0302	0 (0.0%)	1 (0.6%)	0 (0.0%)
0301	37 (33.6%)	57 (34.3%)	71 (33.2%)	1104-0301	1 (0.9%)	1 (0.6%)	0 (0.0%)
0302	0 (0.0%)	8 (4.8%)	11 (5.1%)	1201-0301	0 (0.0%)	0 (0.0%)	3 (1.4%)
0402	5 (4.5%)	13 (7.8%)	15 (7.0%)	1202-0602	0 (0.0%)	0 (0.0%)	1 (0.5%)
0501	25 (22.7%)	36 (21.7%)	51 (23.8%)	1301-0201/2	0 (0.0%)	0 (0.0%)	2 (0.9%)
0502	2 (1.8%)	5 (3.0%)	4 (1.9%)	1301-1302	0 (0.0%)	1 (0.6%)	1 (0.5%)
0503	2 (1.8%)	0 (0.0%)	1 (0.5%)	1301-0602	0 (0.0%)	1 (0.6%)	0 (0.0%)
0602	4 (3.6%)	9 (5.4%)	7 (3.3%)	1301-0603	3 (2.7%)	3 (1.8%)	14 (6.5%)
0603	4 (3.6%)	5 (3.0%)	18 (8.4%)	1302-0201/2	0 (0.0%)	1 (0.6%)	0 (0.0%)
0604	2 (1.8%)	3 (1.8%)	8 (3.7%)	1302-0501	2 (1.8%)	10 (6.0%)	13 (6.1%)
0605	0 (0.0%)	1 (0.6%)	0 (0.0%)	1302-0502	0 (0.0%)	1 (0.6%)	0 (0.0%)
0609	1 (0.9%)	2 (1.2%)	1 (0.5%)	1302-0604	2 (1.8%)	1 (0.6%)	5 (2.3%)
				1302-0605	0 (0.0%)	1 (0.6%)	0 (0.0%)
				1302-0609	1 (0.9%)	2 (1.2%)	1 (0.5%)
				1303-0201/2	0 (0.0%)	1 (0.6%)	1 (0.5%)
				1303-0301	4 (3.6%)	6 (3.6%)	3 (1.4%)
				1304-0301	11 (10.0%)	25 (15.1%)	29 (13.6%)
				1304-0302	0 (0.0%)	1 (0.6%)	0 (0.0%)
				1304-0604	0 (0.0%)	0 (0.0%)	1 (0.5%)
				1322-0603	1 (0.9%)	2 (1.2%)	3 (1.4%)
				1322-0604	0 (0.0%)	1 (0.6%)	0 (0.0%)
				1401-0503	2 (1.8%)	0 (0.0%)	1 (0.5%)
				1503-0301	1 (0.9%)	0 (0.0%)	0 (0.0%)
				1503-0602	2 (1.8%)	0 (0.0%)	1 (0.5%)
				1602-0502	2 (1.8%)	3 (1.8%)	4 (1.9%)

[a] Each subject contributes two alleles (haplotypes) to the analysis, thus, the total number of alleles (haplotypes) in each column is twice the number of subjects. One HPV negative control sample could not be HLA-typed for the *DRB1* locus (it was known to be positive for alleles in the *DRB1*03*, **11*, or **13* groups) and was excluded from the analyses.

haplotypes, whereas 7 (2.9%) were homozygous at the *DRB1* locus, 32 (13.1%) were homozygous at the *DQB1* locus, and the remaining 187 (76.6%) subjects were heterozygous at both the *DRB1* and *DQB1* loci.

Detection of homozygous *DRB1-DQB1* haplotypes was positively associated with invasive cervical cancer (adjusted OR, 5.5, 95% CI, 1.5–20.2), whereas homozygosity for *DRQ1* alone (OR, 1.1; 95% CI, 0.3–3.7) was not significantly associated with risk of cervical cancer. None of the seven subjects homozygous for *DRB1* alone had cervical cancer, although the sample size was too small to determine whether this represented a negative association with cervical cancer.

Among the *a priori* hypothesized associations on which we focused our analysis (*DR11*, *DR13*, *DQ02*, *DQ03*, and

Table 3 Crude and adjusted ORs for the association between cervical carcinoma and *a priori* hypothesized class II HLA alleles and haplotypes

DQB1 and *DRB1* alleles	Cases *vs.* HPV + controls Crude OR (95% CI)	Cases *vs.* HPV − controls Crude OR (95% CI)	Cases *vs.* HPV-controls Adjusted OR[a] (95% CI)	Cases *vs.* combined controls Crude OR (95% CI)	Cases *vs.* combined controls Adjusted OR[a] (95% CI)
*DQB1*0201/2*	1.4 (0.7–2.8)	2.9 (1.3–6.3)	1.1 (0.4–3.1)	2.1 (1.1–3.9)	1.2 (0.5–2.8)
*DQB1*03*	0.6 (0.3–1.2)	0.8 (0.4–1.6)		0.8 (0.4–1.4)	0.8 (0.4–1.9)
*DQB1*0602*	0.5 (0.1–2.2)	0.9 (0.2–3.3)		0.7 (0.2–2.4)	0.6 (0.1–2.7)
*DRB1*13*	0.5 (0.6–1.1)	0.5 (0.3–1.0)	0.5 (0.2–1.4)	0.5 (0.3–1.0)	0.5 (0.2–1.1)
*DRB1*11*	0.8 (0.3–1.7)	0.9 (0.4–1.8)		0.8 (0.4–1.6)	1.0 (0.4–2.2)
*DRB1*1101-DQB1*0301*	1.4 (0.5–3.5)	3.6 (1.2–10.6)	7.4 (1.5–36.5)	2.1 (0.9–4.9)	2.6 (1.0–7.1)

[a] Adjusted for birthplace, no. of live-born infants, age, and other alleles in Table.

DQ06 alleles), we evaluated whether there was a difference in association with cervical cancer among those homozygous for those alleles *versus* those heterozygous for alleles. Using conditional logistic regression analysis to account for the individual matching of cases to controls and to control for potential confounding factors, we found that heterozygosity for *DR13* was somewhat negatively (but not significantly) associated with cervical cancer (OR, 0.5; 95% CI, 0.2–1.3), although none of the nine subjects homozygous for *DR13* had cervical cancer. Neither heterozygosity nor homozygosity for *DR11* and *DQ03* were associated with risk of cervical cancer. *DQ06* was not tested because there were only two subjects in our population that were homozygous for *DQB1*06* alleles. However, those homozygous for *DQB1*02* were significantly more likely to have cervical cancer (adjusted OR, 6.5; 95% CI, 1.1–36.9), whereas heterozygosity for *DQB1*02* did not seem to be associated with cervical cancer risk (adjusted OR, 1.0; 95% CI, 0.4–2.4).

Discussion

Among this group of previously unscreened Senegalese women, we observed *DRB1*1101-DQB1*0301* to be more common among cervical carcinoma cases compared with controls. This association has been reported in previous investigations of British women with CIN (8) and Swedish women with cervical carcinoma who were positive for HPV types other than HPV 16 (9). However, Sanjeevi *et al.* (39), in a similar study among Swedish women, did not find the same haplotype association with CIN among HPV 16-seronegative subjects.

Although we observed an association between cervical carcinoma and a specific *DRB1-DQB1* haplotype containing *DQB1*03*, we were unable to detect an association between the *DQB1*03* allele group and cervical carcinoma found in other studies. Several studies have observed an increase in frequency of *DQB1*03* alleles with dysplasia among Spanish women (15), with CIN among British women (8, 10, 23), and with cervical carcinoma among African-American (17), British (12), German (11), Japanese (13), Norwegian (14), and United States (16, 40) women. One report also found women homozygous for *DQB1*03* were more likely to have CIN than women heterozygous for this allele group (23). However, a number of other studies reported no association between *DQB1*03* alleles and CIN among Europeans (18) and cervical carcinoma among northwest English (24, 41), Brazilian (42), Hispanic (19), French (20), and Swedish (9) women. The findings from our analysis support observations of no association between the *DQB1*03* allele and cervical cancer.

Additionally, despite our observation of an association between a *DRB1*11-DQB1*03* haplotype and cervical carcinoma, we did not observe an association with *DRB1*11* alone. A strong association of *DRB1*11* with CIN was observed among British women (23). Syrjänen *et al.* (21) showed an increase in frequency of *DR5* (*DRB1*11, DRB1*12*) among women with high-grade CIN and women with HPV 16, and al though Glew *et al.* (24) found no significant *HLA* associations among cervical carcinoma patients, they noted that all patients possessing *DR5* had HPV 16-positive tumors.

The first report to observe an increase in frequency of HLA-DR5 antigen among German women with cervical carcinoma suggested the association was attributable to linkage disequilibrium between DR5 and DQw3 (11). Our study suggests that the combination of *DR11* with the *DQB1*0301* allele is associated with cervical carcinoma, because *DRB1*1101* and *DQB1*0301* alleles alone did not show any association. Individually, each allele was observed in similar frequencies among cases and controls (*DRB1*1101*: cases 10.9%, combined controls 9.3%; *DQB1*0301*: cases 33.6%, combined controls 33.7%). However, the *DRB1*11-DQB1*03* haplotype was detected more often among cases than controls. This finding suggests that *DQB1*03* may be a marker, which, when coupled with *DRB1*1101* or another gene in linkage disequilibrium with the *DRB1*1101-DQB1*0301* haplotype, may influence the development of HPV-associated cancers. Our study was able to make this distinction because *DR11* is observed in haplotype combinations with *DQ* alleles other than *DQB1*0301* among African groups, whereas *DR11* is found in linkage disequilibrium with *DQB1*0301* in most other ethnic groups (26). Another analysis of the HLA influence on cervical carcinoma among African-American women (17) only conducted *DQB1* analysis, and thus was unable to test whether particular alleles in linkage disequilibrium have an (interactive) effect on the development of cervical carcinoma. Variations in HLA haplotype distributions among different ethnic populations may explain important differences between studies.

Our study found evidence of a negative association between *DRB1*13* alleles and cervical carcinoma consistent with previous reports (11, 16, 18–22). *DRB1*13* has been found with lower frequency among Hispanic women with cervical carcinoma (OR, 0.3; 95% CI: 0.1–0.7; $P = 0.001$; Ref. 19) and French women (OR, 0.3; $P = 0.0004$; Ref. 20) compared with controls. A decreased cancer risk associated with *DR6* (*DRB1*13* and *DRB1*14*) has also been observed among German (11), Mexican (22), and Finnish women (21). Additionally, a DR13-serotype-associated *DQ* allele (*DQB1*0603*) was found to have a negative association with invasive squamous cell carcinoma of the cervix in a Spanish population (15). Our study extends these previous findings by showing similar results among West African women.

We found that our crude results required adjustment for

place of birth, number of live-born infants, and age, all recognized risk factors for cervical cancer, in addition to factors that we found differed in frequency between the various *HLA* alleles. Additionally, we simultaneously adjusted our estimates for the other *a priori* hypothesized alleles and haplotypes. After adjusting for potentially confounding variables, we did not detect an association between *DQB1*0201/2* and cervical carcinoma in our study population. This contrasts with studies that have observed a decrease in frequency of *DQB1*0201* among African-American (17) and Norwegian women (14) with cervical cancer as compared with controls. However, we did find that homozygosity for *DQB1*02* was positively associated with cervical cancer. We found no associations between *DQB1*06, *0602, *0603,* or **0604* and cervical carcinoma in our study population. Other investigators have observed an increase of *DQB1*0602* (19, 25), *DQB1*0603* (15), *DQB1*0604* (17), and *DQ1* (*DQB1*05* and *DQB1*06*; Ref. 13) among cervical carcinoma patients compared with controls. A number of studies have detected an association between *DQB1*0602* and cervical carcinoma in the *DRB1*1501-DQB1*0602* haplotype (19, 42). Perhaps *DQB1*06* is associated with cervical cancer when it is in linkage disequilibrium with *DRB*15*. Other studies have observed an increased frequency of *DR15* among cervical carcinoma patients (18, 42); although this association remains uncertain, the results of a study by Hildesheim *et al.* (16) found *DRB1*1501-DQB1*0602* to be decreased among CIN patients. We were not able to assess this haplotype association, because our Senegalese population did not contain the *DRB1*1501-DQB1*0602* haplotype.

People homozygous for positively or negatively associated alleles may show a greater predisposition for disease than those heterozygous for such alleles, because the *HLA* locus is responsible for mounting an immune response against infections. Few studies have examined HLA homozygosity as it relates to cervical cancer. Of the studies that have conducted this analysis, one found homozygosity for *DQB1*03* to be positively associated with cervical HPV infection (12), whereas another found homozygosity for *DQB1*0302* to be associated with a higher risk of CIN (16). Our analyses did not support these previous findings.

The *DRB1* and *DQB1* distribution of alleles of the combined controls was similar to that of another HLA study among a Senegalese population (43). Other HLA studies of African and West African populations (44, 45) show similar class II HLA trends (Appendix). *DRB1*1304,* extremely rare in other populations, is the most prevalent *DRB1* allele in these West African populations.

Various HLA alleles have been found at a higher or lower frequency among HPV-positive and HPV-negative control groups. These differences suggest that certain HLA alleles may play a role in the persistence or clearance of HPV. However, because HLA loci *DRB1* and *DQB1* are so polymorphic, there is the potential for a few alleles to differ between the two groups by chance. Several studies have noted no difference in HLA distribution among HPV-positive and HPV-negative controls (19, 39–40). Our comparison supports those observations, suggesting HLA type is not associated with susceptibility, persistence, or detection of HPV infection.

Sixty-four percent of the cervical cancer patients in our study population tested positive for HPV at the time of enrollment into the study. This percentage is lower than reported in most studies of HPV DNA detection in cervical cancer patients (5). However, because of the advanced stages of cancer among many of the cervical carcinoma cases at the time of enrollment, several tissue samples were necrotic and may have been otherwise histologically inadequate for sampling and testing. Recent evidence questions the existence of true HPV negative cervical carcinomas and suggests that inadequate histological sampling, integration of HPV genome into human DNA (46), or disruption of the L1 region of HPV DNA (which is normally used for HPV detection and typing) may account for false-negative or undetectable HPV test results among cervical cancer cases (47, 48).

One of the limitations of our study was the relatively small number of available cervical carcinoma cases. However, even with the small number of cases in this study, we were still able to detect associations reported in previous studies. Additionally, in studies involving multiple comparisons, it is possible that a significant result could be found by chance. The associations reported in our study were based on *a priori* hypotheses.

The postulated role of HLA class II alleles in the risk of cervical neoplasia is in their ability to elaborate proteins that facilitate immune recognition and control of HPV replication. If HLA expression is suppressed, lost, or increased among HPV-infected individuals of a particular HLA type, this may suggest that the ability or inability to ward off HPV infection is related to HLA type. Studies have shown up-regulation of HLA class II expression among women with cervical carcinoma (49–52). HPV infection seems to induce HLA class II expression in cervical squamous epithelium, which is not normally expressed in this region. Furthermore, HLA class II antigens have been observed to be expressed differentially, with DRB expression increased relative to DQB antigens (52, 53). A loss of HLA class I expression has been observed among cervical carcinoma patients (49–52, 54, 55). Recent studies have found altered HLA class I antigen expression can be attributed to specific genetic mutations (55, 56). Understanding the effects of changes in HLA expression subsequent to HPV exposure may explain biological mechanisms in which HPV interacts with HLA related to the development of susceptibility to, or protection against, cervical cancer.

This study of Senegalese women supports previous findings that the HLA haplotype *DRB1*1101-DQB1*0301* is increased among cervical carcinoma cases compared with controls. We were unable to confirm any association between *DQB1*03* and cervical carcinoma, except when coupled with *DRB1*1101*. These observations suggest that specific *DRB1-DQB1* haplotype combinations, rather than individual *DRB1* or *DQB1* alleles, are important in cervical carcinoma susceptibility. Because *DRB1* alleles appear in linkage disequilibrium with different *DQB1* alleles among different ethnic populations, variation in haplotype combinations may help explain the different results observed in previous studies of HLA and cervical carcinoma risk. Our study of Senegalese women confirms and extends findings from previous studies in other ethnic groups showing associations between specific HLA class II molecules and invasive cervical cancer.

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Appendix HLA class II allele and haplotype distribution among Senegalese study population controls in comparison with other HLA-typed West African populations

DRB1 allele	*n*	%	Senegal [43][a]	*DRB1-DQB1* haplotype	*n*	%	Gambia [44][b]	Malawi [44][b]
0101	2	0.5%	0.6%	0101-0501	2	0.5%	DR1-DQ5: 4.1%	4.6%
0102	9	2.4%	0.6%	0102-0501	9	2.4%		
0301	17	4.5%	9.0%	0301-0201/2	16	4.3%	DR0301-DQw2: 2.7%	7.3%
0302	29	7.7%	2.8%	0301-0203	1	0.3%		
0401	2	0.5%		0301-0501	1	0.3%		
0403	3	0.8%	0.4%	0302-0402	28	7.4%	DR0302-DQw4: 3.4%	6.4%
0405	14	3.7%	0.6%	0302-0501	1	0.3%		
0701	13	3.5%	7.5%	0401-0301	1	0.3%	DR4-DQB*0301: 0.1%	0.0%
0801			2.8%	0401-0302	1	0.3%	DR4-DQB1*0302: 4.5%	2.7%
0802			7.2%	0403-0302	3	0.8%		
0803			1.3%	0405-0201/2	3	0.8%	DR4-DQw2: 0.9%	0.3%
0804	15	4.0%		0405-0302	11	2.9%		
0806	4	1.1%		0701-0201/2	12	3.2%	DR7-DQw2: 4.3%	4.3%
0901	14	3.7%	1.4%	0701-0203	1	0.3%		
1001	45	12.0%	4.3%	0804-0301	14	3.7%	DR8-DQw7: 3.0%	4.3%
1101	35	9.3%	9.7%	0804-0604	1	0.3%	DR3-DQw6: 0.3%	0.0%
1102	30	8.0%	6.0%	0806-0602	4	1.1%		
1103			0.2%	0901-0201/2	13	3.5%	DR9-DQw2: 7.1%	10.4%
1104	1	0.3%	0.7%	0901-0203	1	0.3%		
1201	3	0.8%	0.4%	1001-0501	45	12.0%	DR10-DQw5: 8.9%	0.9%
1202	1	0.3%		1101-0203	1	0.3%	DR1101-DQw2: 0.2%	0.0%
1301	23	6.1%	4.3%	1101-0301	18	4.8%	DR1101-DQ0301: 4.2%	6.4%
1302	34	9.0%	4.5%	1101-0501	5	1.3%	DR1101-DQw5: 0.1%	0.0%
1303	11	2.9%	1.5%	1101-0502	1	0.3%		
1304	56	14.9%	27.1%	1101-0602	9	2.4%	DR1101-DQw6: 2.0%	8.5%
1305			0.5%	1101-0604	2	0.5%		
1322	6	1.6%		1102-0301	28	7.4%		
1401	1	0.3%	0.8%	1102-0302	1	0.3%		
1503	1	0.3%		1104-0301	1	0.3%		
1601			1.6%	1201-0301	3	0.8%	DR12-DQ0301: 0.4%	0.0%
1602	7	1.9%		1202-0602	1	0.3%		
Missing	2			1301-0201/2	2	0.5%		
Total (missing)	376			1301-0302	2	0.5%		
DQB1 allele	*n*	%		1301-0602	1	0.3%	DR13-DQw6: 3.6%	4.2%
0201/2	49	13.0%		1301-0603	17	4.5%		
0203	4	1.1%		1302-0201/2	1	0.3%	DR13-DQw2: 0.6%	0.0%
0301	128	33.9%		1302-0501	22	5.9%	DR13-DQw5: 0.3%	0.3%
0302	19	5.0%		1302-0502	1	0.3%	DR1302-DQw1: 16.4%	7.6%
0402	28	7.4%		1302-0604	6	1.6%		
0501	86	22.8%		1302-0605	1	0.3%		
0502	9	2.4%		1302-0609	3	0.8%		
0503	1	0.3%		1303-0201/2	2	0.5%	1303-0301: 2.2%	0.9%
0602	16	4.2%		1303-0301	9	2.4%	1304-0301: 27.3%	3.0%
0603	23	6.1%		1304-0301	54	14.4%		
0604	11	2.9%		1304-0302	1	0.3%		
0605	1	0.3%		1304-0604	1	0.3%		
0609	3	0.8%		1322-0603	5	1.3%		
Missing	0			1322-0604	1	0.3%		
Total (missing)	378			1401-0503	1	0.3%	DR14-DQw1: 0.5%	0.9%
				1503-0301	0	0.0%		
				1503-0602	1	0.3%	DR15-DQw6: 0.8%	13.4%
				1602-0502	7	1.9%	DR16-DQw5: 1.5%	0.3%
				Missing	2			
				Total (missing)	376			

[a] Tiercy et al.: Mandenka (Mandenkalu) of Senegal.
[b] Hill et al: The Gambia (West Africa) and Malawi (South-Central Africa).

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