

## Determinants of Human Papillomavirus Coinfections among Montreal University Students: The Influence of Behavioral and Biologic Factors

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### Abstract

**Background:** Human papillomavirus (HPV) coinfections are common among HPV-infected individuals, but the significance and etiology of these infections remain unclear. Though current evidence suggests that women with coinfections have increased HPV exposure (i.e., more sexual partners), it is also hypothesized that these women may represent a subgroup with increased biologic susceptibility. This study sought to examine determinants of coinfections in a cohort of young women, examining both behavioral and biologic factors related to HPV acquisition over time.

**Methods:** Female university students ( $n = 537$ ) in Montreal, Canada, were followed for 2 years at 6-month intervals. At each visit, cervical specimens were collected for cytology and HPV testing, and women completed a questionnaire about lifestyle and behavior. HLA alleles were typed from purified DNA collected from cervical specimens. Two definitions of coinfections were used: cumulative coinfection over follow-up and concurrent coinfection at each visit. Multiple logistic regression was used to determine predictors of both cumulative and concurrent coinfections using baseline and time-dependent covariates.

**Results:** The most consistent determinant of coinfection occurrence was number of sexual partners, though several genes of the immune response (*HLA-DQB1\*06:02*, *HLA-G\*01:01:03*, and *HLA-G\*01:01:05*) were also identified as significant predictors of cumulative coinfections.

**Conclusions:** HPV coinfections mainly occur due to increased sexual activity, but biologic susceptibility may also be involved in a subset of women. Immunologic factors may put women at greater risk of coinfections over the long term, but short-term risk is almost exclusively driven by modifiable sexual behaviors.

**Impact:** Additional research should continue to further identify immunologic biomarkers of HPV susceptibility. *Cancer Epidemiol Biomarkers Prev*; 23(5); 812–22. ©2014 AACR.

### Introduction

Coinfections with multiple types of human papillomavirus (HPV) are a common occurrence, particularly among young, sexually active women (1–5). Although the significance of these coinfections in the etiology of cervical cancer has been the subject of debate (6–8), there is evidence to suggest that coinfections may be associated with an increased risk of precancerous cervical lesions when compared with infections with only one type of HPV (also referred to as mono-infections; refs. 9–13).

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To date, most studies that have examined determinants of HPV coinfections have largely focused on behavioral factors such as sexual activity, with little attention given to biologic predictors, such as the genetic makeup or immune response of the host (14–19). As such, the role played by biologic susceptibility in the development of coinfections remains somewhat unclear, although indirect evidence supporting this relationship can be seen in the high rates of HPV coinfections observed among HIV-infected individuals (20–22), and among younger women, in which natural immunity to HPV has not yet developed (9, 14, 16, 18, 19). Indeed, despite the definitive role played by sexual activity in determining a woman's exposure to HPV, it has been suggested that the occurrence of HPV coinfections cannot be explained by sexual behaviors alone (8, 14, 23).

The human immune response is affected by a complex interplay of factors, some of which are directly modifiable (e.g., smoking status and nutrient consumption) and some of which are less so (e.g., age and genetic makeup). Of the genetic factors that may be potentially involved in cervical cancer risk, some of the strongest evidence has been found

for genes in the HLA system, as these play a crucial role in immune function and in determining an individual's resistance to infections (24, 25).

The HLA system comprises a cluster of highly polymorphic genes that encode for various cell-surface molecules, which are divided into diverse classes (class Ia, class Ib, and class II; refs. 24, 26). Through their role in antigen presentation, HLA molecules are thought to be critically involved in cell-mediated immune recognition and viral clearance (25, 26), and as such, there is strong biologic plausibility for the HLA system to be involved in the acquisition of HPV coinfections. Indeed, although HLA alleles have been implicated in HPV acquisition (27–29), persistence (28, 30), and carcinogenic progression (24, 26, 31, 32), no studies to date have looked at the impact of specific HLA alleles on the risk of developing HPV coinfections.

At the same time, however, modifiable factors such as smoking status, vegetable consumption, and condom use have also been associated with the occurrence of HPV more generally (33, 34), though studies of these factors in relation to coinfections have been mixed (8, 14–16, 18). As such, the aim of this study was to investigate the influence of both biologic and behavioral predictors on the acquisition of HPV coinfections, with a particular focus on HLA alleles as candidate immune factors. Importantly, because modifiable risk factors may operate at different time scales than factors which are nonmodifiable, we have chosen to examine potential determinants over both short-term and long-term intervals, using multiple definitions of HPV coinfection occurrence.

## Materials and Methods

### Study population and procedures

The McGill–Concordia cohort, a prospective study of the natural history of HPV infection and cervical neoplasia among female university students, has been described in detail elsewhere (35). Recruitment of the cohort took place between November 1996 and January 1999 among female students attending either the McGill or Concordia University health clinics in Montreal, Quebec. Participants were eligible for the study if they planned to remain in Montreal for 2 years and had not been treated for cervical abnormalities in the past year. Eligible participants were required to make five clinic visits in total, returning every 6 months over a 2-year period.

At each visit, samples of endocervical and ectocervical cells were collected using two Accelon cervical biosamplers (Medscand Inc.). A Pap smear was prepared with the first sampler, and the cervical cells collected with the second sampler were used for HPV DNA testing. At enrollment, a self-administered questionnaire was used to collect information on sociodemographics, diet, smoking, alcohol use, reproductive history, medical history, and sexual practices. At each subsequent visit, updated information on all exposures except diet and sociodemographics was collected.

### HPV DNA detection

HPV DNA was purified from the cervical samples using QIAamp columns (Qiagen) and amplification of  $\beta$ -globin DNA with PC04 and GH20 primers was used to verify the integrity of processed DNA. Specimens that were  $\beta$ -globin-positive were further tested for the presence of HPV DNA using a PCR protocol with the L1 consensus primers MY09/MY11 and HMB01. HPV typing of the amplified products was done using a line blot assay (Roche Molecular Systems) for the detection of 27 HPV types (35).

### HLA genotyping

For the sake of analytic efficiency we restricted HLA genotyping to women who met one of two conditions: (i) women who participated in the study for at least 3 visits, or (ii) women who had a persistent infection with the same HPV type for 2 consecutive visits (27). HLA alleles were typed from purified DNA obtained from cervical specimens collected at enrollment, using PCR amplification of sequence-specific primers for *HLA-B\*07*, *DQB1\*03*, *DQB1\*06:02*, *DRB1\*13*, *DRB1\*15:01*, and direct DNA sequence analysis for *E\*01:01*, *E\*01:03*, *G\*01:01:01*, *G\*01:01:02*, *G\*01:01:03*, *G\*01:01:05*, *G\*01:01:07*, *G\*01:01:08*, *G\*01:03*, *G\*01:04:01*, *G\*01:04:03*, and *G\*01:05N*, the details of which can be found elsewhere (27, 28). These HLA types were chosen *a priori* based on consistent published evidence to support their role in susceptibility to both HPV infection (27–30) and cervical cancer (24, 26, 31, 32).

### Statistical analysis

Two definitions of HPV coinfections were used to examine potential predictors at different time points. A cumulative coinfection was defined as 2+ types of HPV detected at any point over the study period, i.e., during a single visit or over the course of multiple visits. By contrast, a concurrent coinfection was limited to the detection of 2+ types of HPV during a single clinic visit, i.e., at the same point in time. As such, we did not distinguish between women who acquired one new HPV infection in addition to a previous infection and women who acquired two new HPV infections concomitantly at a given visit. Coinfection outcomes were not mutually exclusive: a woman with a concurrent coinfection at one visit was also counted as having a cumulative coinfection overall, and would thus contribute to the analyses for each outcome.

Unconditional multivariate logistic regression was used to estimate associations between exposure variables and each coinfection outcome. Separate models were used to contrast women with coinfections to women with mono-infections, and also to women who remained HPV-negative. The reasoning behind this choice was our hypothesis that each comparison might yield different aspects of coinfection etiology. In particular, we hypothesized that the coinfection versus HPV-negative model would be where sexual activity exerted the greatest influence on coinfection occurrence, as these groups of women would likely have the most divergent risk profiles. By

contrast, we hypothesized that sexual activity would be less important in the coinfection versus monoinfection (all HPV<sup>+</sup>) model and that this might be where more subtle determinants of risk (e.g., biologic factors) would emerge.

The choice of which exposures to investigate as potential determinants was made on the basis of the published literature and on the data that were available in our cohort. With the exception of the HLA alleles, all exposure data were obtained from the baseline and follow-up questionnaires described previously. For the HLA alleles, a "dominant" allele approach was used, such that women who were homozygous or heterozygous for a given allele were compared with women in whom the allele was absent.

Potential determinants of cumulative coinfections were examined using exposures assessed at baseline only, to best represent the effects of nonmodifiable risk factors that do not vary over time. Potential determinants of concurrent coinfections were examined using repeated measures models with both baseline and time-dependent exposures as candidate predictors. The purpose of the repeated measures analysis was to explain the presence of a coinfection at a particular visit using data from the time interval just before that visit (~6 months). This approach also allowed us to make full use of the cohort data because each woman could contribute multiple visits to the analysis.

For the model involving repeated measures, the generalized estimating equations (GEE) extension of logistic regression was used to adjust the ORs and SEs for the within-subject correlation between multiple visits by the same individual (36). As it was assumed that observations from visits closer together in time would be more correlated, a first-order autoregressive working correlation matrix was used. Prevalent coinfections detected at enrollment were excluded from this analysis as we felt these infections could be the result of exposures that were not necessarily recent, and which were already captured in the cumulative model. As such, the repeated measures analysis only examined determinants of concurrent coinfections that were newly detected over follow-up.

In the cumulative analysis, we constructed bivariate logistic regression models for baseline exposures and cumulative HPV outcomes. Variables that were significant at  $P < 0.25$  were then considered as candidates for inclusion in the multivariate models. Selection of independent variables into the final models was based on backward elimination using a significance level of  $P < 0.1$ .

After the construction of the cumulative outcome models, significant baseline variables from this analysis were used as a starting point for the GEE models. In addition to the baseline exposures, all variables for which there were time-dependent data were also considered as candidate predictors in this analysis. For a coinfection at visit  $v$ , a time-dependent variable referred to an exposure that occurred in the interval between visit  $v-1$  and visit  $v$ . Final multivariate GEE models were again selected using back-

ward elimination at a significance level of  $P < 0.1$ . All statistical analyses were conducted using SAS Version 9.2.

## Results

Baseline demographic and sexual behavior variables for this cohort have been reported in detail previously (27, 35). The mean age of the cohort was 23 years at enrollment and the vast majority (>80%) of women described themselves as white. HPV infection status did not differ with respect to age, ethnicity, or socioeconomic status, though women who became HPV-positive tended to have been younger at first intercourse, and to have had more lifetime sex partners. At enrollment, nearly half of the women (45%) had 5 or more lifetime sex partners, a proportion that increased to 60% by the end of the study.

After exclusions for missing data on HLA alleles and other covariates of interest, there were 537 women and 2,499 individual visits with complete data for analysis. Sixty-five additional visits were excluded from the concurrent analysis because they contained prevalent coinfections at enrollment (Fig. 1).

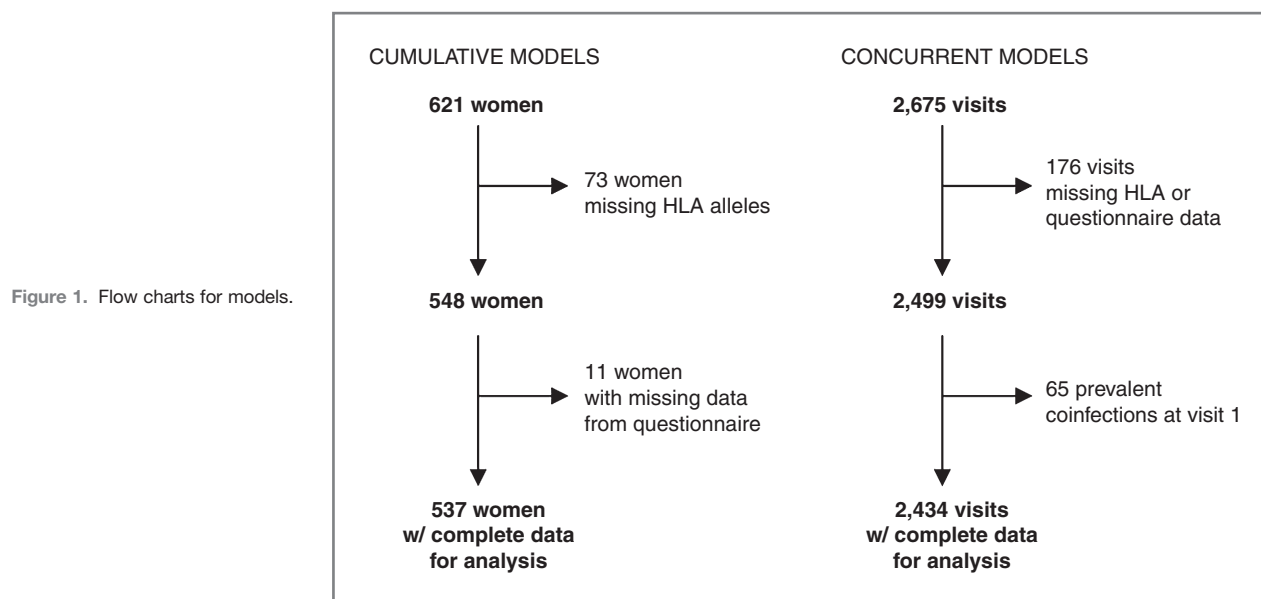
### Cumulative coinfection status: bivariate results

Table 1 shows the bivariate associations between baseline exposures (significant at  $P < 0.25$ ) and cumulative HPV infection status. Of the 537 women, there were 115 (21.4%) with a monoinfection and 192 (35.8%) with a coinfection detected cumulatively over the study. Two hundred and thirty women (42.8%) remained HPV-negative throughout follow-up.

Several strong associations were observed in the coinfection versus HPV-negative model (Table 1). In particular, variables that were significantly associated with occurrence of coinfections included age at first intercourse, number of sex partners (both lifetime and new), history of anal sex, history of yeast infections, history of sexually transmitted infections (STIs; particularly *Chlamydia*), alcohol consumption, and cigarette smoking. Though no associations were found for Black/Hispanic participants, women of Asian descent seemed to be significantly protected compared with white women. In the second bivariate model comparing coinfections with monoinfections, there were far fewer significant associations: only number of new sex partners, frequency of sex per week (inconsistently), and the *HLA-G\*01:01:01* allele were associated with cumulative coinfection occurrence.

### Cumulative coinfection status: multivariate results

Table 2 shows the multivariate associations for all variables retained in the final cumulative models, mutually adjusted for one another. In the first model, compared with women who remained HPV-negative, significant baseline predictors of developing a cumulative coinfection included having  $\geq 2$  lifetime sex partners, having  $\geq 2$  new sex partners in the year before enrollment (OR, 3.87; 95% confidence interval, CI, 2.16–6.93), having a previous history of STIs (OR, 2.96; 95% CI, 1.31–6.69), and having the *HLA-DQB1\*06:02* allele (OR, 1.83; 95% CI, 1.08–3.09).



Being age 27 years or older at enrollment was also significantly protective (OR, 0.34; 95% CI, 0.17–0.67). In the second model, in which all women were HPV-positive, significant predictors of cumulative coinfections included having  $\geq 2$  new sex partners in the last year (OR, 2.74; 95% CI, 1.48–5.08) as well as possessing the *HLA-G\*01:01:05* (OR, 6.44; 95% CI, 1.66–24.95) allele. In contrast, possessing the *HLA-G\*01:01:03* allele was significantly protective (OR, 0.34; 95% CI, 0.14–0.81). Though a very strong association was found for the former allele in particular, it should be noted that this result was based on relatively few women ( $n = 15$ ), due to the rarity of this allele in the cohort (Table 1). In addition, the *DQB1\*06:02* allele was also associated with increased odds of coinfection in this comparison, though it did not reach statistical significance (OR, 1.62; 95% CI, 0.90–2.93).

#### Concurrent coinfection status: multivariate results

In the repeated measures models, the 537 subjects from the cumulative analysis generated 2,434 visits in which incident coinfection status could be assessed. Of these, there were 746 HPV-positive visits (30.6%) and 266 visits (10.9%) with concurrent coinfections detected. Table 3 presents the results of the multivariate repeated measures models for predictors of concurrent coinfections at individual visits. These results differed slightly from the cumulative models, though age and number of sex partners were still the most important determinants of coinfection, particularly when visits with coinfections were contrasted with HPV-negative visits.

In terms of the time-dependent exposures, aspects of sexual behavior seemed to be the most important factors associated with concurrent coinfections in both models. In particular, number of new sex partners, irregular use of oral contraceptives (OCs), and never using condoms were all significantly associated with coinfection occur-

rence in at least one of the models. Number of new sex partners was the only significant predictor found in both models, in which an effect was seen for having any new sex partner in the last 12 months. Women who reported never using condoms since their previous visit were also more likely to have coinfections detected in both comparisons, although the associations were only borderline significant. Notably, irregular use of OCs was also positively associated with coinfection occurrence (OR, 1.88; 95% CI, 1.08–3.26) but only in comparison with women with mono-infections. Though age at first intercourse and frequency of sex per week were both significant at  $P < 0.1$ , none of the CIs were significantly different from unity and no clear trend seemed to be evident for these variables.

#### Discussion

In this study, we investigated both behavioral and biologic predictors of HPV coinfection occurrence in a longitudinal cohort of young women. To gain the greatest amount of information from this analysis, we used two different comparison groups and two definitions of coinfections, and examined both baseline and time-varying risk factors. In the models that included HPV-negative women, we expected the most important predictors of coinfections to be predominantly related to sexual activity, and so these models served as a good comparison with the exclusively HPV-positive models, in which we hypothesized that sexual factors might be less important. Though some studies have reported increased sexual activity to be the main explanation for coinfection occurrence (15, 37), there has been little research into biologic factors that may also be involved in modulating individual susceptibilities. By including several exposures purported to affect the immune response (i.e., HLA alleles, smoking status, and vegetable consumption), we

**Table 1.** Associations between baseline exposures (significant at  $P < 0.25$ ) and cumulative HPV infection status

| Variable                                 | Frequency (%)                         |  |                                      | OR (95% CI)                     |                                  |
|--|---------------------------------------|--|--------------------------------------|---------------------------------|----------------------------------|
|  | HPV-negative<br><i>n</i> = 230 (42.8) | Monoinfection<br><i>n</i> = 115 (21.4) | Coinfection<br><i>n</i> = 192 (35.8) | Coinfection vs.<br>HPV-negative | Coinfection vs.<br>monoinfection |
| Age at enrollment, y                     |                                       |  |                                      |                                 |                                  |
| 17–20                                    | 96 (45.1)                             | 42 (19.7)                              | 75 (35.2)                            | 1.00                            | 1.00                             |
| 21–23                                    | 61 (37.9)                             | 34 (21.1)                              | 66 (41.0)                            | 1.39 (0.87–2.20)                | 1.09 (0.62–1.90)                 |
| 24–26                                    | 32 (37.2)                             | 21 (24.4)                              | 33 (38.4)                            | 1.32 (0.75–2.34)                | 0.88 (0.45–1.71)                 |
| ≥27                                      | 41 (53.2)                             | 18 (23.4)                              | 18 (23.4)                            | 0.56 (0.30–1.06)                | 0.56 (0.26–1.19)                 |
| Race                                     |                                       |  |                                      |                                 |                                  |
| White                                    | 178 (40.7)                            | 93 (21.3)                              | 166 (38.0)                           | 1.00                            | 1.00                             |
| Black/Hispanic <sup>a</sup>              | 19 (45.2)                             | 8 (19.1)                               | 15 (35.7)                            | 0.85 (0.42–1.72)                | 1.05 (0.43–2.57)                 |
| Asian                                    | 33 (56.9)                             | 14 (24.1)                              | 11 (19.0)                            | <b>0.36 (0.18–0.73)</b>         | 0.44 (0.19–1.01)                 |
| Age at first intercourse, y              |                                       |  |                                      |                                 |                                  |
| <16                                      | 40 (33.3)                             | 26 (21.7)                              | 54 (45.0)                            | 1.00                            | 1.00                             |
| 16–18                                    | 121 (43.4)                            | 59 (21.1)                              | 99 (35.5)                            | <b>0.61 (0.37–0.99)</b>         | 0.81 (0.46–1.43)                 |
| ≥19                                      | 69 (50.0)                             | 30 (21.7)                              | 39 (28.3)                            | <b>0.42 (0.24–0.74)</b>         | 0.63 (0.32–1.22)                 |
| Number of lifetime sex partners          |                                       |  |                                      |                                 |                                  |
| 0–1                                      | 75 (65.8)                             | 19 (16.7)                              | 20 (17.5)                            | 1.00                            | 1.00                             |
| 2–4                                      | 76 (44.2)                             | 36 (20.9)                              | 60 (34.9)                            | <b>2.96 (1.63–5.39)</b>         | 1.58 (0.75–3.36)                 |
| 5–9                                      | 48 (33.6)                             | 30 (21.0)                              | 65 (45.4)                            | <b>5.08 (2.74–9.43)</b>         | 2.06 (0.96–4.41)                 |
| ≥10                                      | 31 (28.7)                             | 30 (27.8)                              | 47 (43.5)                            | <b>5.69 (2.91–11.11)</b>        | 1.49 (0.68–3.24)                 |
| Number of new sex partners <sup>b</sup>  |                                       |  |                                      |                                 |                                  |
| 0  | 120 (52.9)                            | 54 (23.8)                              | 53 (23.3)                            | 1.00                            | 1.00                             |
| 1  | 78 (47.9)                             | 31 (19.0)                              | 54 (33.1)                            | 1.57 (0.98–2.52)                | 1.78 (0.99–3.18)                 |
| ≥2                                       | 32 (21.8)                             | 30 (20.4)                              | 85 (57.8)                            | <b>6.01 (3.58–10.11)</b>        | <b>2.89 (1.64–5.07)</b>          |
| Anal sex <sup>c</sup>                    |                                       |  |                                      |                                 |                                  |
| Never                                    | 182 (45.7)                            | 83 (20.8)                              | 133 (33.4)                           | 1.00                            | 1.00                             |
| Ever                                     | 43 (34.5)                             | 32 (23.0)                              | 59 (42.5)                            | <b>1.68 (1.08–2.62)</b>         | 1.15 (0.69–1.92)                 |
| Oral contraceptive use <sup>b</sup>      |                                       |  |                                      |                                 |                                  |
| Regularly                                | 156 (42.9)                            | 83 (22.8)                              | 125 (34.3)                           | 1.00                            | 1.00                             |
| Sometimes                                | 21 (36.2)                             | 10 (17.2)                              | 27 (46.6)                            | 1.61 (0.87–2.97)                | 1.79 (0.82–3.90)                 |
| Never                                    | 53 (46.1)                             | 22 (19.1)                              | 40 (34.8)                            | 0.94 (0.59–1.51)                | 1.21 (0.67–2.18)                 |
| Frequency of sex/week <sup>b</sup>       |                                       |  |                                      |                                 |                                  |
| <1 time                                  | 52 (39.1)                             | 24 (18.1)                              | 57 (42.9)                            | 1.00                            | 1.00                             |
| 1–3 times                                | 127 (44.4)                            | 70 (24.5)                              | 89 (31.1)                            | 0.64 (0.40–1.02)                | <b>0.54 (0.30–0.95)</b>          |
| 4–6 times                                | 40 (40.8)                             | 18 (18.3)                              | 40 (40.8)                            | 0.91 (0.51–1.63)                | 0.94 (0.45–1.95)                 |
| ≥7 times                                 | 11 (55.0)                             | 3 (15.0)                               | 6 (30.0)                             | 0.50 (0.17–1.44)                | 0.84 (0.19–3.65)                 |
| History of yeast infections <sup>c</sup> |                                       |  |                                      |                                 |                                  |
| No                                       | 126 (47.9)                            | 53 (20.2)                              | 84 (31.9)                            | 1.00                            | 1.00                             |
| Yes                                      | 104 (38.0)                            | 62 (22.6)                              | 108 (39.4)                           | <b>1.56 (1.06–2.29)</b>         | 1.10 (0.69–1.75)                 |
| History of STIs <sup>c,d</sup>           |                                       |  |                                      |                                 |                                  |
| No                                       | 219 (44.9)                            | 101 (20.7)                             | 168 (34.4)                           | 1.00                            | 1.00                             |
| Yes                                      | 11 (22.4)                             | 14 (28.6)                              | 24 (49.0)                            | <b>2.84 (1.36–5.97)</b>         | 1.03 (0.51–2.08)                 |
| Chlamydia <sup>c</sup>                   |                                       |  |                                      |                                 |                                  |
| No                                       | 226 (44.3)                            | 106 (20.8)                             | 178 (34.9)                           | 1.00                            | 1.00                             |
| Yes                                      | 4 (14.8)                              | 9 (33.3)                               | 14 (51.9)                            | <b>4.44 (1.44–13.74)</b>        | 0.93 (0.39–2.21)                 |
| Herpes (HSV-2) <sup>c</sup>              |                                       |  |                                      |                                 |                                  |
| No                                       | 226 (43.4)                            | 112 (21.5)                             | 183 (35.1)                           | 1.00                            | 1.00                             |
| Yes                                      | 4 (25.0)                              | 3 (18.8)                               | 9 (56.3)                             | 2.78 (0.84–9.17)                | 1.84 (0.49–6.92)                 |

(Continued on the following page)

**Table 1.** Associations between baseline exposures (significant at  $P < 0.25$ ) and cumulative HPV infection status (Cont'd)

| Variable   | Frequency (%)                         |  |                                      | OR (95% CI)                     |                                  |
|--|---------------------------------------|--|--------------------------------------|---------------------------------|----------------------------------|
|  | HPV-negative<br><i>n</i> = 230 (42.8) | Monoinfection<br><i>n</i> = 115 (21.4) | Coinfection<br><i>n</i> = 192 (35.8) | Coinfection vs.<br>HPV-negative | Coinfection vs.<br>monoinfection |
| Vegetable consumption <sup>c</sup>                 |                                       |  |                                      |                                 |                                  |
| ≥1 serving per day                                 | 47 (45.2)                             | 21 (20.2)                              | 36 (34.6)                            | 1.00                            | 1.00                             |
| >1 serving per week                                | 103 (40.7)                            | 51 (20.2)                              | 99 (39.1)                            | 1.26 (0.75–2.10)                | 1.13 (0.60–2.14)                 |
| 1 serving per week                                 | 64 (44.1)                             | 33 (22.8)                              | 48 (33.1)                            | 0.98 (0.55–1.74)                | 0.85 (0.42–1.70)                 |
| Rarely   | 16 (45.7)                             | 10 (28.6)                              | 9 (25.7)                             | 0.73 (0.29–1.85)                | 0.53 (0.18–1.50)                 |
| Alcohol consumption <sup>e</sup> (drinks per week) |                                       |  |                                      |                                 |                                  |
| 0  | 95 (48.7)                             | 35 (18.0)                              | 65 (33.3)                            | 1.00                            | 1.00                             |
| 1–3  | 77 (49.0)                             | 31 (19.8)                              | 49 (31.2)                            | 0.93 (0.58–1.50)                | 0.85 (0.46–1.57)                 |
| ≥4   | 58 (31.4)                             | 49 (26.5)                              | 78 (42.2)                            | <b>1.97 (1.24–3.13)</b>         | 0.86 (0.50–1.48)                 |
| Average cigarettes smoked/day <sup>c</sup>         |                                       |  |                                      |                                 |                                  |
| Nonsmoker  | 152 (48.0)                            | 66 (20.8)                              | 99 (31.2)                            | 1.00                            | 1.00                             |
| <1   | 10 (43.5)                             | 6 (26.1)                               | 7 (30.4)                             | 1.08 (0.40–2.92)                | 0.78 (0.25–2.42)                 |
| 1–5  | 34 (37.8)                             | 17 (18.9)                              | 39 (43.3)                            | <b>1.76 (1.04–2.98)</b>         | 1.53 (0.80–2.93)                 |
| >5   | 34 (37.8)                             | 26 (24.3)                              | 47 (43.9)                            | <b>2.12 (1.28–3.53)</b>         | 1.21 (0.68–2.13)                 |
| Any  | 78 (35.5)                             | 49 (22.3)                              | 93 (42.3)                            | <b>1.83 (1.24–2.71)</b>         | 1.27 (0.79–2.02)                 |
| HLA-DQB1*03  |                                       |  |                                      |                                 |                                  |
| No   | 70 (37.0)                             | 44 (23.3)                              | 75 (39.7)                            | 1.00                            | 1.00                             |
| Yes  | 160 (46.0)                            | 71 (20.4)                              | 117 (33.6)                           | 0.68 (0.46–1.02)                | 0.97 (0.60–1.55)                 |
| HLA-DQB1*06:02                                     |                                       |  |                                      |                                 |                                  |
| No   | 187 (44.3)                            | 92 (21.8)                              | 143 (33.9)                           | 1.00                            | 1.00                             |
| Yes  | 43 (37.4)                             | 23 (20.0)                              | 49 (42.6)                            | 1.49 (0.94–2.37)                | 1.37 (0.78–2.40)                 |
| HLA-DRB1*15:01                                     |                                       |  |                                      |                                 |                                  |
| No   | 176 (44.2)                            | 87 (21.9)                              | 135 (33.9)                           | 1.00                            | 1.00                             |
| Yes  | 54 (38.9)                             | 28 (20.1)                              | 57 (41.0)                            | 1.38 (0.89–2.13)                | 1.31 (0.78–2.22)                 |
| HLA-G*01:01:01                                     |                                       |  |                                      |                                 |                                  |
| No   | 71 (41.3)                             | 46 (26.7)                              | 55 (32.0)                            | 1.00                            | 1.00                             |
| Yes  | 159 (43.6)                            | 69 (18.9)                              | 137 (37.5)                           | 1.11 (0.73–1.69)                | <b>1.66 (1.02–2.70)</b>          |
| HLA-G*01:01:03                                     |                                       |  |                                      |                                 |                                  |
| No   | 205 (43.8)                            | 94 (20.1)                              | 169 (36.1)                           | 1.00                            | 1.00                             |
| Yes  | 25 (36.2)                             | 21 (30.4)                              | 23 (33.3)                            | 1.12 (0.61–2.04)                | 0.61 (0.32–1.16)                 |
| HLA-G*01:01:05                                     |                                       |  |                                      |                                 |                                  |
| No   | 216 (42.9)                            | 110 (21.9)                             | 177 (35.2)                           | 1.00                            | 1.00                             |
| Yes  | 14 (41.2)                             | 5 (14.7)                               | 15 (44.1)                            | 1.31 (0.62–2.78)                | 1.86 (0.66–5.27)                 |
| HLA-G*01:01:07                                     |                                       |  |                                      |                                 |                                  |
| No   | 221 (42.9)                            | 108 (21.0)                             | 186 (36.1)                           | 1.00                            | 1.00                             |
| Yes  | 9 (40.9)                              | 7 (31.8)                               | 6 (27.3)                             | 0.79 (0.28–2.27)                | 0.50 (0.16–1.52)                 |

NOTE: Bold indicates statistical significance based on the 95% confidence interval.

<sup>a</sup>These categories combined due to small numbers.

<sup>b</sup>In the last year before enrollment.

<sup>c</sup>In lifetime.

<sup>d</sup>Includes trichomoniasis, chlamydia, herpes, syphilis, gonorrhea, and genital sores.

<sup>e</sup>In the past 5 years.

hoped to tease out some of these more subtle predictors of coinfection occurrence.

As expected, in the final multivariate models examining predictors of cumulative coinfections, we found several differences in the two comparison models, each revealing slightly different aspects of coinfection etiology. As hypothesized, the coinfection versus HPV-nega-

tive model mainly reflected traditional risk factors for HPV infection (e.g., age) as well as markers of increased sexual activity (e.g., more lifetime sex partners and history of STIs). Number of new sex partners was the only significant predictor of coinfections that was present in both models, likely because it is a proxy for exposure to new types of HPV. Notably, three HLA alleles were

**Table 2.** Multivariate models with independent predictors of cumulative HPV coinfection

| Variable                                | Cumulative coinfection<br>vs. HPV-negative ( <i>n</i> = 422)<br>OR <sup>a</sup> (95% CI) | Cumulative coinfection<br>vs. mono-infection ( <i>n</i> = 307)<br>OR <sup>a</sup> (95% CI) |
|---|--|--|
| Age at enrollment                       |  |  |
| ≤26                                     | 1.00   | 1.00   |
| ≥27                                     | <b>0.34 (0.17–0.67)</b>  | 0.54 (0.25–1.17)   |
| Number of lifetime sex partners         |  |  |
| 0–1                                     | 1.00   | 1.00   |
| 2–4                                     | <b>2.15 (1.14–4.08)</b>  | 1.19 (0.54–2.65)   |
| ≥5                                      | <b>3.62 (1.88–6.95)</b>  | 1.22 (0.56–2.66)   |
| Number of new sex partners <sup>b</sup> |  |  |
| 0                                       | 1.00   | 1.00   |
| 1                                       | 1.33 (0.80–2.21)   | 1.57 (0.85–2.89)   |
| ≥2                                      | <b>3.87 (2.16–6.93)</b>  | <b>2.74 (1.48–5.08)</b>  |
| History of STIs <sup>c</sup>            |  |  |
| No                                      | 1.00   | 1.00   |
| Yes                                     | <b>2.96 (1.31–6.69)</b>  | 1.06 (0.50–2.24)   |
| HLA-DQB1*06:02                          |  |  |
| No                                      | 1.00   | 1.00   |
| Yes                                     | <b>1.83 (1.08–3.09)</b>  | 1.62 (0.90–2.93)   |
| HLA-G*01:01:03                          |  |  |
| No                                      | 1.00   | 1.00   |
| Yes                                     | 1.12 (0.47–2.71)   | <b>0.34 (0.14–0.81)</b>  |
| HLA-G*01:01:05                          |  |  |
| No                                      | 1.00   | 1.00   |
| Yes                                     | 1.06 (0.35–3.21)   | <b>6.44 (1.66–24.95)</b>   |

NOTE: Bold indicates statistical significance based on the 95% confidence interval.

<sup>a</sup>Mutually adjusted for all other variables in a column.

<sup>b</sup>In the year before enrollment.

<sup>c</sup>In lifetime; includes trichomoniasis, chlamydia, herpes, syphilis, gonorrhea, and genital sores.

retained as significant predictors in the final cumulative models, and seemed to be particularly associated with coinfections among HPV-positive women. In an effort to investigate whether there might be greater frailty among women who acquired higher numbers of coinfections over follow-up, we reran our coinfection versus mono-infection model postanalysis comparing women who acquired 2 to 3 types (*n* = 131) and 4+ types (*n* = 61) with those who only acquired only 1 type over follow-up (*n* = 115). Although most of the variables did not change markedly, we did observe a striking effect for *HLA-G\*01:01:05* among women who acquired 4+ types, as the strength of the association increased considerably: OR, 11.01; 95% CI, 1.63–74.34 (data not shown). Though this effect was only based on 6 women due to the rarity of this allele in the cohort, this finding bolsters the notion that certain HLA alleles could mediate susceptibility to coinfections with many HPV types. Future studies with larger sample sizes should attempt to investigate this relationship further.

In the repeated measures models, the results were generally similar to the cumulative models, though we had the added benefit of assessing predictors that could

vary over time. As such, we identified several time-dependent predictors of concurrent coinfections, namely, new sex partners, condom use, and OC use. Women who reported "never" using condoms since their previous visit had significantly increased odds of concurrent coinfection in both of the repeated measures models, whereas irregular (i.e., "sometimes") use of OCs since the previous visit was a significant predictor of concurrent coinfection among HPV-positive women only. Though we restricted this analysis to coinfections that were newly detected, an important caveat of these models is that we were not able to distinguish between newly acquired HPV infections and reactivation of latent infections that women may have had previously. Although there is undoubtedly some reactivation taking place in our cohort, the strong relationship we found for new sex partners in particular suggests that the occurrence of coinfections is being largely driven by new HPV acquisition.

In terms of biologic factors, we found 3 HLA alleles to be significantly associated with coinfections in the models based on cumulative HPV status. As we had hypothesized, the effect was strongest in the all HPV<sup>+</sup> model

**Table 3.** Multivariate models with independent predictors of concurrent HPV coinfection

| Variable                                | Concurrent coinfection<br>vs. HPV-negative<br>(n = 1,954 visits)<br>OR <sup>a</sup> (95% CI) | Concurrent coinfection<br>vs. monoinfection<br>(n = 746 visits)<br>OR <sup>a</sup> (95% CI) |
|---|--|---|
| Baseline exposures                      |  |   |
| Age at enrollment                       |  |   |
| ≤26                                     | 1.00   | 1.00  |
| ≥27                                     | <b>0.38 (0.17–0.87)</b>  | 0.54 (0.24–1.25)  |
| Age at first intercourse                |  |   |
| <16                                     | 1.00   | 1.00  |
| 16–18                                   | 1.21 (0.76–1.94)   | 1.60 (0.97–2.63)  |
| ≥19                                     | 1.10 (0.58–2.06)   | 1.18 (0.61–2.27)  |
| Number of lifetime sex partners         |  |   |
| 0–1                                     | 1.00   | 1.00  |
| 2–4                                     | <b>3.13 (1.60–6.14)</b>  | 1.12 (0.55–2.27)  |
| ≥5                                      | <b>4.90 (2.38–10.10)</b>   | 1.07 (0.51–2.25)  |
| Time-dependent exposures                |  |   |
| Number of new sex partners <sup>b</sup> |  |   |
| 0                                       | 1.00   | 1.00  |
| 1                                       | <b>1.64 (1.22–2.20)</b>  | <b>1.59 (1.09–1.34)</b>   |
| ≥2                                      | <b>2.07 (1.53–2.81)</b>  | <b>1.52 (1.03–2.24)</b>   |
| Frequency of sex per week <sup>c</sup>  |  |   |
| <1 time                                 | 1.00   | 1.00  |
| 1–3 times                               | 1.00 (0.75–1.34)   | 0.93 (0.62–1.38)  |
| 4–6 times                               | 1.34 (0.95–1.88)   | 0.85 (0.50–1.44)  |
| 7+ times                                | 0.96 (0.54–1.67)   | 0.43 (0.15–1.21)  |
| Oral contraceptive use <sup>c</sup>     |  |   |
| Regularly                               | 1.00   | 1.00  |
| Sometimes                               | 1.26 (0.83–1.90)   | <b>1.88 (1.08–3.26)</b>   |
| Never                                   | 0.98 (0.73–1.32)   | 1.23 (0.83–1.82)  |
| Condom use <sup>c</sup>                 |  |   |
| Regularly                               | 1.00   | 1.00  |
| Sometimes                               | 1.19 (0.91–1.56)   | 1.24 (0.83–1.83)  |
| Never                                   | <b>1.33 (1.01–1.74)</b>  | 1.56 (1.00–2.44)  |

NOTE: Bold indicates statistical significance based on the 95% confidence interval.

<sup>a</sup>Mutually adjusted for all other variables in a column.

<sup>b</sup>Since the last 2 visits (~12 months).

<sup>c</sup>Since the last visit (~6 months).

(in which HPV infection status was effectively "controlled for"), and only after adjustment for number of new sexual partners. As the HLA system is critical to the host immune response (25), it is conceivable that these alleles may play an important role in both immune recognition and clearance of the virus. Indeed, because coinfections themselves are a product of both HPV acquisition and persistence (i.e., the longer an infection persists, the greater the chance it will be detected and overlap with a new infection), it is possible that these HLA alleles may be operating at several critical points in the course of infection. Indeed, HLA-G acts as negative regulator of the immune response by preventing antigen recognition and T-cell migration, as well as through suppression of T and natural killer cells' cyto-

toxic effects. Thus, the immunosuppressive properties of HLA-G may contribute to both the susceptibility and persistence of HPV infections (38).

As this is the first study to explicitly explore the role of HLA alleles in the development of HPV coinfections, it is somewhat difficult to contextualize these results with other studies in the literature. Nevertheless, the *HLA-DQB1\*06:02* allele has been linked to both cervical cancer and malignant precursor lesions in several studies (24, 26, 39), and in a previous study in this cohort, the allele was associated with both high-risk HPV and HPV16 positivity, though the associations did not reach statistical significance (27). Another previous study in this cohort also found the *HLA-G\*01:01:02* allele to be associated with both increased cumulative risk and persistence of HPV16



(28), and this allele was also recently linked to invasive cervical cancer (32).

Both the *HLA-G\*01:01:03* and *HLA-G\*01:01:05* alleles associated with coinfections here were also previously found to be associated with increased odds of certain low-risk HPV infections in this cohort, but not among women purported to be highly exposed to HPV (28). The DNA sequences of the *HLA-G\*01:01:03* and *HLA-G\*01:01:05* alleles are almost identical except for the presence of a synonymous mutation (proline) at codon 57 in the *HLA-G\*01:01:03* allele. Because the polymorphism in codon 57 does not change the HLA-G amino acid composition and presumably its function, it is difficult to deduce the mechanism by which a silent mutation could have a direct influence on susceptibility to coinfections at this time. However, this mutation is in the vicinity of Glu-63, which interacts with the P2 position of loaded peptide (40). Therefore, it is possible that this mutation could affect the loading of viral peptides and consequently influence cell-mediated immune responses.

The results of our repeated measures analysis are also noteworthy because conflicting results have been found for condom use and OC use in other studies of coinfections. For instance, though none of the longitudinal studies of coinfections have identified condom use as a significant predictor, it was associated with decreased occurrence of coinfections in two cross-sectional studies (18, 41). Although condom use has not been found to have the consistent protective effect with HPV that it has with other STIs (42, 43), it is likely that when used properly, condoms convey at least some degree of protection, either by acting as a protective barrier against skin-to-skin transmission or by decreasing HPV viral load (34). OC use has also been found to have an equivocal effect on coinfection risk (15, 16); however, it is also strongly associated with sexual activity, and residual confounding may exist even after adjusting for number of sexual partners (44, 45). Similarly, it is worth noting that sexual factors such as condom use and OC use may also play a role in HPV susceptibility, through modifications to the cervical immune environment. In particular, recent research has shown that rapid changes in female sex hormones as well as exposure to seminal fluid (through noncondom use) can have profound effects on the cervical microenvironment and immune response (46).

### Strengths and Conclusions

The main strengths of this study were its longitudinal, repeated measures design and the availability of high-quality biologic and behavioral data on much of our cohort. This allowed us to examine a wide range of potential determinants of HPV coinfections and to assess their relative effect(s) by controlling for other relevant exposures. The repeated measures design also allowed for the assessment of determinants that vary over time, which may better represent temporal etiologic associations. We were limited, however, by the rarity of certain HLA

alleles, which resulted in some findings being based on relatively small numbers. Nevertheless, this research adds to the current understanding of HPV coinfection occurrence, and provides some of the first evidence of the role played by biologic susceptibility in an otherwise healthy cohort of young women.

In conclusion, in this study assessing predictors of HPV coinfections in a population of female university students, we have confirmed that coinfections are caused by a complex interplay of factors affecting both exposure and susceptibility to HPV. In particular, we identified multiple markers of increased sexual activity and poor contraceptive practices as modifiable risk factors for coinfection occurrence, and we found several HLA alleles to be significant predictors of coinfections, even after controlling for more traditional risk factors such as age and sexual activity. Additional research is needed to further identify and pinpoint immunologic biomarkers of HPV susceptibility, particularly among women who acquire many types of HPV over short periods of time, as these women may represent an important risk subgroup for further cervical disease.

### Disclosure of Potential Conflicts of Interest

P.-P. Tellier received a commercial research grant from Merck, commercial research support from Purdue Pharma, and honoraria from the speakers bureau of Bayer's. F. Coutlée has provided expert testimony for Merck Sharp Dome. E.L. Franco is a consultant/advisory board member of Merck and Roche. No potential conflicts of interest were disclosed by the other authors.

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