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Bacterial Vaginosis and Risk of HIV Infection in the Context of *CD101* Gene Variation

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Background: Whether bacterial vaginosis (BV) and *CD101* immunoglobulin-like (Ig-like) variants independently increase HIV risk through mucosal inflammation is not well understood. We evaluated whether the impact of BV on HIV acquisition in women differs by the presence or absence of candidate *CD101* Ig-like variants.

Methods: We used data from 2 studies of HIV serodiscordant couples in east (Kenya, Tanzania, and Uganda) and southern (Botswana, South Africa, and Zambia) Africa, which longitudinally assessed HIV acquisition (by ELISA) and BV (by Nugent score ≥7). We used previously generated *CD101* sequence data for each case and control participant to create a binary variable indicating the presence/absence of any of 5 *CD101* Ig-like variants.

Results: Confirming previously shown results in this cohort, Ig-like variants increased HIV-infection risk (adjusted hazard ratio [aHR], = 2.63; 95% confidence interval [CI], 1.41 to 4.89). BV was associated with 2.5-fold higher HIV-infection risk only in the absence of Ig-like variants (aHR = 2.47; 95% CI, 0.99 to 6.15; $P = 0.052$), whereas in the presence of Ig-like variants, BV was not associated with higher HIV-infection risk (aHR = 0.87; 95% CI, 0.35 to 2.15; $P = 0.765$); however, a test for interaction was nonsignificant ($P = 0.116$).

Conclusions: We hypothesized that both BV and *CD101* Ig-like variants facilitate HIV acquisition by augmenting similar genital inflammation pathways. Our findings indicate that inflammatory mucosal effects of Ig-like variants may influence the impact of BV on HIV risk. Host-defined inflammatory pathways may be useful targets for HIV prevention.

Key Words: BV, *CD101*, inflammation, HIV

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INTRODUCTION

Bacterial vaginosis (BV) is a vaginal dysbiosis that is highly prevalent among women, particularly in sub-Saharan Africa.[1] Analyses in several large cohorts and a meta-analysis[2–4] have found that BV is associated with an increased risk of HIV acquisition in women. Genital mucosal inflammation is a key underlying factor in proposed mechanisms through which BV increases HIV risk. For instance, genital mucosal inflammation has been linked to increased $CD4^+$ T cells, which serve as targets for HIV infection when activated,[5] and different genital microbiota have been associated with different levels of mucosal inflammatory response.[6] It is therefore important to explore additional mechanisms through which genital inflammation impacts HIV risk.

Recently, we demonstrated that a cluster of missense variants located in the immunoglobulin-like (Ig-like) domains of the *CD101* gene are associated with a 4-fold increased risk of heterosexually acquired HIV among HIV-exposed Africans.[7] CD101 is a potential marker for activated mucosal tissue resident memory T cells[8] and is also thought to modulate regulatory T-cell suppression of tissue inflammation.[9,10] Given this documented relevance of CD101 for the regulation of mucosal inflammation and the association of *CD101* variants with risk of HIV acquisition, we evaluated whether the impact of BV on HIV acquisition in women differs by the presence or absence of candidate *CD101* Ig-like variants.

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METHODS

Study Population and Design

We used data from 2 studies of HIV serodiscordant couples in east (Kenya, Tanzania, and Uganda) and southern (Botswana, South Africa, and Zambia) Africa: the Partners Preexposure Prophylaxis Study (Partners PrEP) with monthly follow-up,[11] and the Partners in Prevention HSV/HIV Transmission Study (Partners HSV2) with quarterly follow-up.[12] In our case–cohort design, we included all HIV-seroconverting cases with a sample available for genotyping. Non-HIV—seroconverting controls were selected for genotyping as those determined to have been exposed to HIV based on epidemiological quantification of HIV exposure.[7,13] Only women who were HIV negative at enrollment, randomized to the placebo arm (Partners PrEP), had *CD101* genotyping data available, and had baseline risk scores were included in the analysis.

Ethical Considerations

The Partners PrEP and Partners HSV2 protocols were approved by the University of Washington Human Subjects Review Committee and ethical review committees at each of the relevant study sites. All study participants provided written informed consent.

Data Collection

HIV Infection and BV

Longitudinal HIV infection was ascertained by ELISA/Western blot serology or by real-time polymerase chain reaction. BV was longitudinally assessed by Nugent score such that Nugent score 7–10 and 0–6 indicated "BV" and "no BV," respectively. To ensure that BV exposure preceded the infection outcome, BV was lagged for each participant over follow-up. For example, for association of the HIV outcome at a given visit, we evaluated the Nugent score at the prior visit.

CD101 Variants

We used previously generated *CD101* sequence data (as described in Ref. 7) for each case and control participant to characterize 20 *CD101* functional variants (including mis-sense, 5′- and 3′-untranslated region, and splice site). We created a binary variable indicating the presence/absence of any of 5 *CD101* Ig-like variants: *rs3754112*, *rs17235773*, *rs116063197*, *rs34882009*, and *rs12093834*.

Baseline Risk Scores

We used validated scores quantifying baseline HIV acquisition risk for HIV serodiscordant couples developed from multivariate modeling of baseline variables, including age of the HIV-1–uninfected partner, married and/or cohabiting partnership, number of children, unprotected sex, uncircumcised male HIV-1–uninfected partner, and plasma HIV-1 RNA in the HIV-1–infected partner.[13]

Statistical Analyses

We compared baseline characteristics by HIV-infection status using the χ^2 test. For the selected controls, we estimated a post hoc probability of selection for genotyping within stratification defined by baseline exposure risk score[13] and study (Partners PrEP or Partners HSV2). Using these estimated selection probabilities, and adjusting for potential confounders (age, region [east Africa vs. southern Africa], and longitudinal quantification of any self-reported unprotected sex with the study partner), we assessed the association between HIV infection and each exposure (BV and *CD101* Ig-like variants) using 2-stage estimation methods for case–cohort designs.[14]

To assess for effect modification, we fit a confounder adjusted-model including the main terms and interaction terms between BV and *CD101* Ig-like variants. For sensitivity analyses, we explored effect modification of BV/HIV-infection association with 2 other categories of *CD101* variation (cytoplasmic variants [*rs12097758*, *rs12067543*, *rs34248572*, *rs150494742*, *X1_117576709*] and UTR/splice site variants [*rs2296448*, *X1_117578861*, *rs35163967*]) that were not previously found to be significantly associated with HIV-acquisition risk.[7] We used the *survey* package in R statistical software version 3.6.1 and SAS version 9.4 (SAS Institute, Inc., Cary, NC) for all analyses and evaluated significance at $\alpha = 0.05$.

RESULTS

In our analyses, 561 women with a median age of 29 years (interquartile range, 24–35 years) contributed 6743 observations. The maximum number of observations per woman was 39, with a range of 2–13, and 2–39 observations per woman in the Partners HSV2 and Partners PrEP studies, respectively. Overall, 54 women (9.6%) became HIV-infected during follow-up (cases), 170 women (20 cases and 150 controls) had BV at baseline, and 197 (29 cases and 168 controls) had at least 1 *CD101* Ig-like variant. Comparing HIV-infected with HIV-uninfected women, there were no differences by study and region. However, those who became HIV infected were more likely to report having unprotected sex at baseline (30% vs. 16%; $P = 0.017$) and have higher median baseline risk scores (6 vs. 5; $P = 0.007$) than those who remained HIV uninfected (Table 1).

BV was not significantly associated with risk of HIV infection (adjusted hazard ratio [aHR] = 1.53; 95% confidence interval [CI], 0.82 to 2.84), but having at least 1 *CD101* Ig-like variant was significantly associated with higher risk of HIV infection (aHR = 2.63; 95% CI, 1.41 to 4.89; $P = 0.002$) (Table 2).

BV was marginally associated with about 2.5-fold higher risk of HIV infection among those without an Ig-like variant (aHR = 2.47; 95% CI, 0.99 to 6.15; $P = 0.052$), but there was no association of BV with HIV-infection risk among those with a *CD101* Ig-like variant (aHR = 0.87; 95% CI: 0.35 to 2.15; $P = 0.765$) (Table 2). The test for effect modification of the BV–HIV association by *CD101* Ig-like variants was not significant ($P = 0.12$). We also found no evidence of effect modification in sensitivity analyses with non-Ig–like functional variants of *CD101* (cytoplasmic and 5′/3′-UTR variants) (results not shown).

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TABLE 1. Participant Characteristics by HIV Status

Variable	HIV Infected (n = 54), N (%)	HIV Uninfected (n = 507), N (%)
Age in years		
≤24	14 (25.9)	135 (26.6)
25–29	23 (42.6)	143 (28.2)
30–34	6 (11.1)	116 (22.9)
35–39	7 (13.0)	76 (15.0)
≥40	4 (7.4)	37 (7.3)
Study*		
Partners HSV2	28 (51.9)	272 (53.6)
Partners PrEP	26 (48.1)	235 (46.4)
Region		
Eastern	49 (90.7)	481 (94.9)
Southern	5 (9.3)	26 (5.1)
CD101 Ig-like variant†	29 (53.7)	168 (33.1)
Any unprotected sex with study partner†	16 (29.6)	80 (15.8)
Baseline risk score†		
0-3	6 (11.1)	113 (22.3)
4	5 (9.3)	91 (17.9)
5	12 (22.2)	77 (15.2)
6+	31 (57.4)	226 (44.6)

N (%), number (percent).
*Partners HSV2 = the Partners in Prevention HSV/HIV Transmission Study; Partners PrEP = Partners Preexposure Prophylaxis Study.
†P-value < 0.05.

DISCUSSION

We evaluated the relationship between BV and HIV-acquisition risk stratified by whether a woman has 1 or more *CD101* Ig-like variants. There was a marked difference in the association of BV with HIV acquisition among women with Ig-like variants vs. those without Ig-like variants, although the test for interaction between BV and *CD101* Ig-like variants did not reach statistical significance. Assessed independently, BV was not associated with HIV risk. However, having an Ig-like variant increased HIV risk 2.6-fold. The results from our stratified analyses identified an association of BV with HIV in the absence of Ig-like variants. This suggests a possible role for *CD101* variation in modifying mucosal inflammation independent of microbiota; not evaluating this host genetic effect could explain the wide variation in point estimates across studies assessing the relationship between BV and HIV acquisition. Moreover, given this effect, we conclude that future evaluations for determinants of mucosal inflammation include evaluation of *CD101* variation.

Although CD101 may have pleomorphic effects in its referent form, one documented function is to enhance the capacity of regulatory T cells to suppress mucosal inflammation.[9,10] We interpret our results as consistent with *CD101* Ig-like variants deleteriously impacting this function, leading to reduced activity of regulatory T cells. The resulting increase in mucosal inflammation, even in the absence of external stimuli (such as BV), mediates increased risk of HIV. In the context of Ig-like variants, we found no evidence that the additional tissue-resident inflammation generated in response to BV augments HIV-infection risk. In the absence of *CD101* Ig-like variant-associated inflammation, BV tended to augment risk of HIV infection. This concept of a host-encoded inflammation set point conveying increased HIV risk is the counterpoint of previous studies of "immune quiescence" that has been linked to natural resistance to HIV[15]; however, to date, there have not been studies of *CD101* variation in populations with natural resistance to HIV.

A limitation of our study is that the number of HIV infections among those with BV was small, which decreased the power of our study to detect an interaction effect; only 7 cases (13%) had both BV and Ig-like variants. Also, of note is that our analysis focused on cohorts that had previously contributed to identifying and replicating the relationship of Ig-like variation with HIV acquisition. Furthermore, we did

TABLE 2. Exposure Distribution and Associations With HIV Infection

Exposure	HIV Infected, N (%)	HIV Uninfected, N (%)	Total	Unadjusted HR (95% CI); *P*	aHR (95% CI); *P*	*P* for whether HRs Differ
BV						
BV Positive	18 (33.3)	1628 (24.3)	1646	1.64 (0.90 to 3.00); 0.106	1.53 (0.82 to 2.84); 0.179	NA
BV negative	36 (66.7)	5061 (75.7)	5097	Ref	Ref	
Ig-like variants						
Overall						
Ig-like variant+	29 (53.7)	2424 (36.2)	2453	2.38 (1.29 to 4.40); 0.006	2.63 (1.41 to 4.89); 0.002	NA
Ig-like variant−	25 (46.3)	4265 (63.8)	4290	Ref	Ref	
Interaction model						
Ig-like variant+						0.116
BV positive*	7 (24.1)	669 (27.6)	676	NA	0.87 (0.35 to 2.15); 0.765	
BV negative*	22 (75.9)	1755 (72.4)	1777	NA	Ref	
Ig-like variant−						
BV positive*	11 (44.0)	959 (22.5)	970	NA	2.47 (0.99 to 6.15); 0.052	
BV negative*	14 (56.0)	3306 (77.5)	3320	NA	Ref	

N (%), number of observations (percent); aHR, adjusted hazard ratio (adjusted for age, region, and any unprotected sex with study partner); Ref, reference.
*Denominator is number of observations in subgroup (overall row).

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not evaluate microbiological community-state types more generally to assess whether *CD101* variation may mediate inflammatory impact through altered mucosal microbiota. Further studies are needed to address these issues and to evaluate these gene–phenotype associations to better understand the generalizability of these findings and gain insight on their underlying mechanisms. For example, among women without BV, highly prevalent *Lactobacillus*-deficient bacterial communities have been associated with increased induction of mucosal HIV target cells,[6] highlighting the existence of mechanisms involving specific microbial communities that may modify HIV risk in women.

Our findings demonstrate that host-defined inflammation pathways may play an important role in increasing HIV acquisition risk. Specifically, *CD101* Ig-like variation may be a useful target for pharmacologic intervention as host-directed drugs for HIV prevention.

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