HLA Class I and II alleles, heterozygosity and HLA-KIR interactions are associated with rates of genital HSV shedding and lesions

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Abstract

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Variation at HLA and KIR loci is associated with the severity of viral infections. To assess associations of genital HSV-2 infection with human HLA and KIR genetic loci, we measured the frequencies of genital HSV DNA detection and of genital lesions in HSV-2 seropositive persons. We followed 267 HSV-2 seropositive persons who collected daily genital swabs and recorded lesions for 30 days. All persons were laboratory- documented as HIV-seronegative, and all were Caucasian by self-report. HSV detection rate and lesion frequency were compared by genotype using Poisson regression. Overall, HSV was detected on 19.1% of days and lesions on 11.6% of days. The presence of HLA-A*01 was directly associated with HSV detection frequency while the presence of HLA-C*12 was inversely associated with HSV detection frequency. The presence of HLA-A*01 was directly associated with lesion rate, while HLA-A*26, -C*01 and -DQB1*0106 were associated with decreased lesions. We observed an interaction between the absence of both 2DS4del and HLA-Bw4 and higher lesion rate. Heterozygosity of HLA was also associated with reduced lesion frequency. Immune control of genital HSV infection relies on multiple interacting immunogenetic elements, including epistatic interactions between HLA and KIR.

Keywords

HLA; KIR; genital herpes; viral shedding; immunogenetics

Introduction

Herpes simplex virus infections have a wide spectrum of clinical and virological severity. The determinants of the phenotype are poorly understood but both host and viral factors likely play a role. In contrast to rare genetic defects that result in severe primary infection (1), host factors controlling severity during the chronic phase of infection are incompletely characterized.

Extensive HSV disease observed in persons with impaired cellular immunity, and reduction of HLA class I on the surface of HSV-infected cells mediated by viral immune evasion genes, suggest that HLA types could influence severity, as they do in HIV infection and other chronic infections. The restriction of both CD8 and CD4 T-cells by HLA molecules suggests that HLA type could also influence the immunogenicity and efficacy of HSV vaccines. Since human NK cells recognize HSV-infected cells *in vitro* and NK deficiency has been associated with severe HSV infections (2), variations in NK cell receptor genotype could also influence severity. Indeed, epistatic associations between HLA alleles, KIR receptors, and the severity of viral infections have been elucidated by several authors (summarized by Bashirova et al.(3)).

We have characterized the clinical and virological severity of genital HSV-2 infection in a cohort of healthy persons who prospectively collected daily swabs of genital secretions and maintained a diary of genital lesions (4). Uniquely amongst studies of HLA-HSV severity correlations available to date, we measure HSV severity using both subject-reported HSV recurrence rates and HSV recurrence objectively detected by intensive mucocutaneous sampling and sensitive PCR. To understand the role of host genetics within the spectrum of HSV-2 infection in this cohort, we genotyped selected HLA class I and II and killer

immunoglobin-like receptors (KIR) loci and examined their associations with the severity of HSV-2 disease.

Results

We included 267 persons who provided a median of 59 (range 30-168) daily swabs of genital secretions for HSV DNA PCR, and a median of 62 (range 30-208) days on which lesions were ascertained (Table 1). The median age was 41 years (range 20-76), and 60% of participants were women. As this analysis was limited to Caucasian participants, 44 persons who self-reported as non-Caucasian and were otherwise eligible were included in the clinical and laboratory protocol, but excluded from the present analysis. All included participants were HSV-2 seropositive and 42% were also HSV-1 seropositive. Thirty-six (13%) were in the first year since HSV-2 acquisition, 174 (65%) had acquired more than 1 year ago, and 57 (21%) had no known acquisition date (that is, had acquired HSV-2 asymptomatically). KIR typing was available on 240 (90%) of the 267 participants. HLA typing was performed by sequencing to the four-digit allele resolution on 207 (78%) to 241 (90%) of the 267 participants, depending on the HLA locus, with 222 persons having four-digit typing for at least 4 of the 5 loci studied. Characteristics of the subset of 240 persons with KIR data and the subset of 222 persons with four-digit HLA typing were both similar to the full cohort, with both subsets including 14% in their first year since acquisition.

Overall, HSV was detected on 3,141 of 16,440 days (19.1%) on which genital swabs were obtained (18.8% of days in the subset with KIR data; n=240 participants). Similarly, genital herpes lesions were noted by the participants on 2,128 of 18,356 (11.6%) of the diary days (11.5% within the KIR subset). Per-session shedding and lesion rates were strongly associated (Spearman's correlation 0.63, p<0.0001).

Distribution of HLA alleles, linkage disequilibrium and KIR genotypes

Fifty-six HLA allelic variants defined at the four-digit (amino acid) level were present in at least 8 persons (Supplemental table 1). After consolidation to the two-digit level, 53 alleles were present in at least 8 persons each (Table 2). Linkage disequilibrium between many pairs of alleles at different loci was observed, for example, with alleles HLA-A*01, -B*08 and -C*07 clustering together (r>0.2 for each pair) and HLA-A*26, -B*38 and -C*12 also moderately associated (r>0.3 for each pair, Supplemental Figure 1). These associations motivated multivariate analysis to address potential confounding between related alleles.

HLA alleles and genital HSV shedding

Within the diploid human genome each participant could have between 5 and 10 unique HLA alleles at the loci studied. Heterozygosity was present in 74% to 88% participants for each locus. Persons with 5 or 6 unique alleles appeared to have an increased shedding rate, although it was not statistically significant (RR=1.1 for each additional homozygous loci, 95% CI 0.9-1.2, p=0.34, Figure 1a).

Of 53 alleles evaluated, 6 alleles were at least marginally associated at p<0.10 with shedding frequency. Separately, the presences of HLA-A*01 (RR=1.5, 95% CI 1.2-1.9, p<0.001), -B*08 (RR=1.4, 95% CI 1.1-1.7, p=0.014), and -C*07 (RR=1.3, 95% CI 1.0-169, p=0.042)

were associated with increased shedding, while HLA-A*26 (RR=0.5, 95% CI 0.3-1.0, p=0.055), -B*38 (RR=0.3, 95% CI 0.1-1.0, p=0.049) and -C*12 (RR=0.5, 95% CI 0.3-0.9, p=0.014) were associated with decreased shedding (Figure 2a). When analyses were restricted to the 222 persons with HLA typing performed to the four-digit level, we confirmed the HLA-A*01 association with higher shedding using the HLA-A*0101 allele designation (RR = 1.5, 9% CI 1.2-2.0, p<0.001). Presence of HLA-B*0801 was also associated with higher shedding frequency (RR=1.4, 95% CI 1.1-1.9, p=0.015) as was HLA-C*0701 (RR = 1.4, 95% CI 1.1-1.8, p=0.016). The decreased shedding associated with HLA-C*12 was confirmed for HLA-C*1203 (RR=0.6, 95% CI 0.3-1.0, p=0.049). HLA-DRB1*1104, which had not been noted when consolidated at the two-digit level, was marginally associated with decreased shedding (RR=0.4, 95% CI 0.2-1.0, p=0.051).

After a separate multiplicity adjustment for each of the 5 HLA alleles identified in univariate analysis, only presence of HLA-A*01 remained significantly associated with genital HSV shedding (p=0.004). Similarly at the four-digit level, only HLA-A*0101 retained significance (p=0.005). In a multivariate model initially including HLA-A*01, -A*26, -B*08, -B*38, -C*07 and -C*12 and adjusted for age, gender, HSV-1 co-infection, and time since acquisition, the presence of the HLA-A*01 allele increased the risk of HSV genital shedding (RR=1.5 (95% CI 1.2-1.9, p=0.001) while the presence of HLA-C*12 decreased it (RR=0.6 (95% CI 0.3-0.9, p=0.027). Cox regression results were similar though HLA-A*26 also remained significantly associated with reduced shedding multivariately. Additionally, the HLA-A*01, -B*08, -DRB1*03 ancestral haplotype (8.1AH) (5) was found in 24 persons and was associated with increased HSV shedding frequency (RR=1.6, 95% CI 1.2-2.2, p=0.005). No additional associations were found with the other HLA grouping categories investigated (Table 2b).

HLA alleles and genital lesion rate

The number of unique HLA alleles at the five loci studied was inversely associated with lesion rate (Figure 1b), with an overall lesion rate of 10.2% among 112 persons heterozygous at all 5 HLA loci and 22.0% among 8 persons having only 5 or 6 unique HLA alleles. The predicted lesion rate increased 17% on a relative scale for each additional homozygous loci (95% CI 1% to 34%, p=0.031). Separately, homozygosity solely at the HLA-B locus was associated with increased lesion frequency (RR=1.5, 95% CI 1.0-2.2, p=0.052) as was homozygosity at the HLA-DRB1 locus (RR=1.6, 95% CI 1.0-2.5, p=0.030). The other loci were not individually significantly associated: HLA-A (p=0.96), HLA-C (p=0.16), and HLA-DQB1 (p=0.18). After adjustment for age, gender, duration of infection and HSV-1 serostatus, HLA-A*01, -C*12 and -DQB1*06 were univariately associated with genital lesion rate (Figure 2b). Having any copies of HLA-A*01 alleles was associated with a RR of 1.5 (95% CI 1.1-2.1, p=0.008) for genital lesion rate. Presence of HLA-C*12 showed an inverse association with lesion frequency, with a RR of 0.4 (95% CI 0.2-0.8, p=0.019). Within HLA class II, presence of HLA-DQB1*06 was protective against lesions, RR 0.7 (95% CI 0.5-0.9, p=0.027). When analyses were repeated for persons with four-digit HLA typing available, HLA-A*0101 (RR=1.6, 95% CI 1.1-2.3, p=0.013) and HLA-C*1502 (RR=2.2, 95% CI 1.0-4.9, p=0.049) were significantly associated with lesion rate, and a few others were marginally significant as well: HLA-C*0102 (RR=0.4, 95% CI

0.1, 1.0, p=0.058), HLA-C*1203 (RR =0.4, 95% CI 0.2-1.1, p=0.064) and HLA-DRB1*1104 (RR=0.2, 95% CI 0.0-1.2, p=0.070).

Univariately, after multiplicity adjustment, no HLA allele remained significantly associated with genital lesion rate. In multivariate analysis originally including HLA-A*01, -A*26, -C*01, -C*12, -DQB1*06, and -DRB1*15, HLA-A*01 remained associated with increased lesion rate (RR 1.6, 95% CI 1.1-2.2, p=0.009) and three other alleles with decreased rate: HLA-A*26 (RR 0.3, 95% CI 0.1-0.9, p=0.037), HLA-C*01 (RR 0.4, 95% CI 0.2-1.0, p=0.048), and HLA-DQB1*06 (RR 0.7, 95% CI 0.5-0.9, p=0.017). In a multivariate Cox regression model, HLAC*12 was selected over -A*26, but inference was otherwise similar. Presence of the HLA-A*01, -B*08, -DRB1*03 ancestral haplotype (8.1AH) was also associated with lesion rate (RR=1.6, 95% CI 1.0-2.6, p=0.037) but no additional associations were found with HLA grouping categories (Table 2b).

KIR types as modifiers between HLA and shedding or lesions rate

We examined potential interactions between HLA and KIR types and genital HSV shedding or lesion rate. Prior to multiple comparison adjustment, we detected five significant associations between combinations of KIR and HLA alleles and either shedding or lesion frequency (Table 3). As supported by Cox regression analysis, having both HLA C group 1 (or lack of HLA C group 2) and a KIR-3DS1 receptor was associated with higher shedding and lesion frequencies. But only one association remained significant following multiple comparison adjustment by HLA allele: the absence of both KIR-2DS4del and HLA-Bw4 was associated with a higher predicted lesion rate (25% of days with lesions) versus any other allelic combination group (8-10% of days with lesions, p=0.0016 for interaction, figure 3). However, the effect seems to be largely driven by two persons in that group of nine with neither 2DS4del nor HLA-Bw4 who have especially high lesion rates (above 60%). Removal of either one of those persons brings the interaction significance level to p=0.021 or to p=0.084, depending on which person is removed.

Classification and Regression Trees

Tree analysis confirmed association between HLA-A*01 and shedding rate (Figure 4), and demonstrated additional associations with symptomatic acquisition, HLA-DQB1*04 and -B*18. A similar tree was built with lesion rate as an outcome, with only symptomatic acquisition was associated with higher lesion rate, as previously described (4, 6). Overall, the proportion of variability in shedding rate explained by HLA measures along with gender, length of infection and HSV-1 coinfection was 13%.

Discussion

In this large cohort of clinically and virologically characterized persons, we found that HLA-A*01 and -C*12 were positively and negatively associated with higher rate of HSV detection, respectively. HLA-A*01 was also associated with a higher rate of lesion frequency, while -A*26, -C*01 and -DQB1*0106 were negatively associated with this measure of severity. Some of these alleles are in linkage disequilibrium and it is not always possible to isolate the influence of particular alleles in a haplotype. We found an epistatic

interaction between KIR-2DS4del allelic variant and HLA-Bw4, with most severe disease among individuals having neither the KIR-2DS4del variant or any copies of HLA-Bw4. This association was based on a few persons, however, and should be confirmed in an independent cohort. The decrease in expected lesion rate with increasing numbers of unique HLA alleles indicates that heterozygosity, independent of any particular allele, may aid in control of disease.

Host genetics, virus strain variation, inoculum size, and the environment, including past infections, likely influence the severity of herpesviruses infections. This targeted host genetic study was motivated by work indicating that T-cells are critical for the control of HSV-2 infection in humans and the fact that genetic variation within HLA controls T-cell immunity. Severe T-cell deficiency, for example in advanced HIV-1-infection, is associated with increases in HSV-2 shedding and lesion rates (7). HSV has evolved immune evasion mechanisms that specifically target both CD8 and CD4 T-cells, consistent with a host-beneficial antiviral response by these effector cells (8, 9).

Particular strengths of this report include assessment of disease severity both through virologic measures (HSV DNA detection on genital swabs) and clinical measures (participant-noted lesions). The large cohort and the large numbers of samples collected per person increased precision of associations and their generalizability to the Caucasian HSV-2 seropositive population. Some of the strongest associations observed for HLA and HSV shedding were also detected for these same genetic markers and rates of lesion frequency, strengthening the validity of our findings. Genetic associations which are not consistent between both ways of measuring herpes severity (shedding frequency and lesion frequency) may reflect different underlying mechanisms of association, or may be a function of limited sampling to assess sporadic outcomes.

Previous studies have correlated HLA type or SNPs in the HLA region with the presence or severity of HSV infection (10-15). Resistance to initial infection and control of established HSV infection likely involve different genetic loci. For example, variation in TLR3 pathway genes have been conclusively linked to fatal primary HSV infection but not to severity in the chronic phase (16). Conversely, HLA loci are clearly associated with the severity of established HIV-1 and HCV infection and of HCV clearance, but have not been associated with susceptibility to initial infection (17, 18). Therefore, we focus review on studies of severity amongst HSV infected persons. Moraru *et al.* did not find associations between HLA and orolabial HSV-1 disease severity in 164 persons with self-reported sporadic vs. recurrent cold sores in Spain (11). Lekstrom-Himes *et al.* studied genital herpes severity in 91 HSV-2-infected persons with a self-reported dichotomous classification of frequent symptomatic vs. asymptomatic infection, using serologic HLA typing (10). In that study, HLA-B*27 and -Cw2 were marginally associated with asymptomatic infection. Our findings extend the work of Seppanen *et al.* (14), who associated HLA homozygosity and oral HSV severity amongst infected persons. To our knowledge, the current report is the first to use viral shedding of HSV-2 as the infection phenotype.

KIR genotyping was included because NK cells contribute to HSV control (2), HLA and KIR cooperate to control NK and possibly also T-cell responses, and because KIR and

HLA/KIR combined genotypes show strong association with outcomes in other human viral infections such as HIV-1 and HCV (3). Previous studies of KIR in HSV have been limited. KIR-2DS2 and KIR-2DL2 were both more commonly found in persons presenting with oral herpetic lesions relative to asymptomatic controls (19), as was the combination of HLA-C*01 with KIR-2DL2 (11). A study of 15 persons found no difference in the level of KIR expression by CD56 (+) NK cells between persons who had frequent HSV-2 recurrences relative to HSV-2 seropositive controls (20).

The biological mechanism(s) for the genotype-phenotype associations we observed are not known. Knowledge of the CD8 T-cell epitope landscape of HSV-2 is incomplete (21), so it is difficult to evaluate potential HLA-specific recognition gaps in the HSV-2 proteome. A limited number of the 77 HSV open reading frames have been thoroughly studied for T cell epitopes in the HSV-1 or HSV-2 context, with a subset of the data applicable to both viral types due to sequence identity or similarity. Disease severity associations have been reported for epitope-specific responses and inhibitory molecule expression, and in some cases HLA transgenic animals and vaccination/challenge models have reinforced the human correlative findings (22-25). Unfortunately, not all of the HLA variants implicated in the present or previous studies are available in HLA transgenic animal models. HLA class I loci implicate CD8 T-cell, while class II variation classically implies a role for CD4 T-cells, albeit exceptions to these mechanistic associations have recently been recognized (26). As some KIR loci are deleted on some chromosomes, have allelic variation when present, and have complex protein expression patterns that may not be predicted by genotype, investigations in this area are particularly complex. T-cells can also express and be modulated through KIR, such that variant KIR loci could influence both T and NK cell activation (27). We do know that HSV-specific CD8 T-cells restricted by certain HLA alleles, namely HLA-B*5701 and -B*2705, are relatively resistant to regulatory T-cell influences, thus demonstrating in principle allele-specific qualitative differences in T cell function (28). HSV has evolved several mechanisms to down-regulate HLA class I protein at the cell surface, and HLA class I molecules can differ in their susceptibility to these viral immune evasion mechanisms (8, 29, 30).

Other genetic loci that have been reported to correlate with HSV-2 and/or HSV-1 infection severity include mannose-binding lectin (31), toll-like receptors (TLRs) (32, 33), *APOE* alleles (34), *TBX21* (35), and a region on chromosome 21 (36). Except for the *APOE* and *TLR* studies, viral shedding rates were not reported. Additional genetic loci within the extended HLA region on chromosome 6, aside from classical HLA class I and II, have also been shown to influence HSV severity, including an allelic variant at SNP rs3130297 genotype in the HLA class II region between the 3' UTR of *BTNL2* and the 5' UTR of *NOTCH4* (13). Several complement protein genes are also located in the HLA major histocompatibility complex (MHC) region. Homozygous null alleles for *C4A* or the isotype gene *C4B* has been reported in severe herpes labialis due to HSV-1 (15).

Our study is limited by having insufficient participants to examine 29 of the 82 observed HLA allelic variants. However, these rare types were observed in 7 or fewer of the 267 participants (<3%). In order to avoid identifying allelic associations that were actually due to other genetic variations associated with race, we excluded study participants who did not

self-report being Caucasian; and thus additional research will be required for establishing severity correlates in other populations. We were concerned that variation in genes such as *MBL2* that show race-associated differences in allele frequency and that have been shown to be associated with infection severity could confound our results (37-39). Self-reported ancestry can be imperfect and future research can potentially include genetic assessment of the background of the participants. We were unable to take a haplotype mapping approach to evaluate the HLA associations we found here, because this requires a binary outcome (40) and we have previous shown dichotomization of HSV rates over repeated sampling to be unreliable and context-dependent (41).

In summary, the present report is the largest study of HLA and HSV-2 associations to date and the first to include objective measures of virologic shedding. Our findings confirm the hypothesis that infection severity phenotypes can correlate with variation in the HLA locus and imply that therapeutic vaccines focusing on T-cell responses may vary in efficacy depending on host immunogenetics. Likely, combined and detailed studies of both host and pathogen genetics will be required to fully determine the mechanisms underlying the large variations in disease phenotype exhibited by genital HSV-2 infection.

Materials and Methods

Subjects and specimens

Participants were enrolled at the University of Washington Virology Research Clinic in Seattle, WA, and Westover Heights Clinic, Portland, Oregon. Entry criteria included age 18 or older, laboratory documented HSV-2 seropositivity and HIV seronegativity, , and general good health. Non-Caucasian persons were enrolled but excluded from the present analysis as race is known to correlate with the frequency distribution of allele frequencies at HLA and other loci. During the time of shedding frequency assessment, the participants did not receive antiviral therapy. We also assessed known correlates of HSV-2 severity: sex, age, presence of HSV-1 infection, and time since initial HSV-2 infection (4).

Participants were instructed to perform a swab of genital secretions daily for HSV PCR and record genital lesions (4). Prior work has shown excellent correlation between clinician and participant obtained samples for HSV detection (42). All persons gave informed written consent and the research protocols were approved by the University of Washington Human Subjects Review Committee.

Laboratory Methods

HSV-1 and HSV-2 serostatus were determined by University of Washington type-specific immunoblot (43) and HIV-1 antibody status was assessed by ELISA. HSV DNA was detected by PCR as previously described (44, 45). Host DNA was prepared from blood by (Paxgene, Qiagen, Valencia, CA) and used for HLA typing, performed by sequencing to four-digit (amino acid level) precision (46). For some subjects, HLA typing was done to two-digit precision using sequence specific oligonucleotide probes at the Puget Sound Blood Center. KIR typing was performed using a sequence-specific primer kit (Dynal, now Life Technologies, Carlsbad, CA) per the manufacturer's instructions.

Statistical Methods

Linkage disequilibrium between HLA types was assessed by computing the r statistic(47, 48). Poisson regression with overdispersion was used to examine association between outcomes and 1) the number of unique alleles and 2) the presence of each specific HLA genotype. Outcomes included shedding rate, defined as frequency of detection of HSV DNA in genital swabs, and lesion rate. Each regression included adjustment for other variables shown previously to be associated with virologic and clinical outcomes: age of the participant, sex, HSV-1 co-infection, and time since genital HSV-2 acquisition (within 1 year, more than 1 year, or unknown) (4) These measures were included a priori to avoid confounding, and backward elimination was *not* performed. Within each HLA locus (HLA-A, -B, -C, -DQB1 and -DRB1), adjustment was made for multiple comparisons using the method of Li and Ji (49, 50). As a sensitivity analysis, Cox regression analyses were performed examining potential HLA and KIR effects using shedding rate (or lesion rate) as the "time" outcome and implementing a failure indicator of "1" for all persons. Where this method contradicted Poisson regression findings, these are noted in the Results section. With 267 persons, we have 80% power to detect differences in outcome rates as low as 12% for HLA types prevalent at 10% or higher.

Multivariate Poisson regression was performed including all measures initially significant at $p < 0.1$ and using backward elimination to create a parsimonious model using all 5 HLA loci. Additional analyses were performed using classification and regression trees with cross validation as a complement to standard regression techniques, in order to allow interactions between potentially influential measures (51, 52). Specific allelic combinations having precedents in the literature were also considered (Table 2b).

KIR/HLA interactions for which each of the four possible combinations of HLA and KIR presence (any copies versus none) was found in at least 5 persons were assessed for associations with clinical outcomes; combinations previously reported by others were considered, if present in sufficient number in our cohort (3, 5, 53, 54). This adjustment procedure was repeated using HLA typing with four-digit precision as well. Software used includes SAS 9.3 (Cary, NC) and R version 2.15.1 ('genetics' package).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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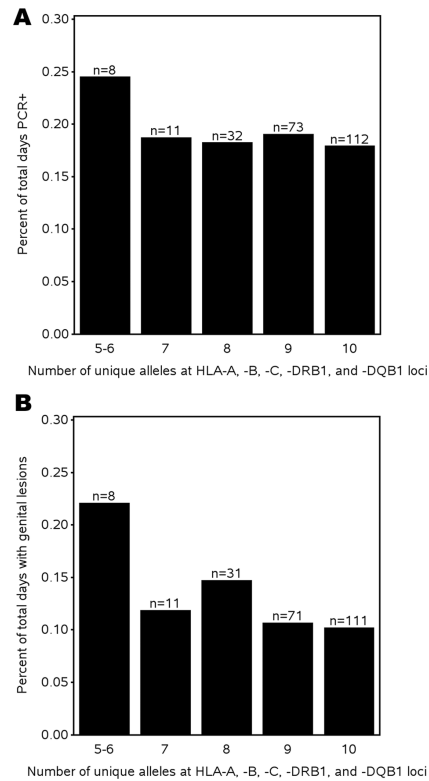
Figure 1.
a: Relationship between number of unique HLA alleles at the A, B, C, DRB1, and DQB1 loci and genital HSV shedding. b: Relationship between number of unique HLA alleles at the A, B, C, DRB1, and DQB1 loci and genital lesion rate. The number of study participants with each number of unique HLA alleles is shown above each bar.
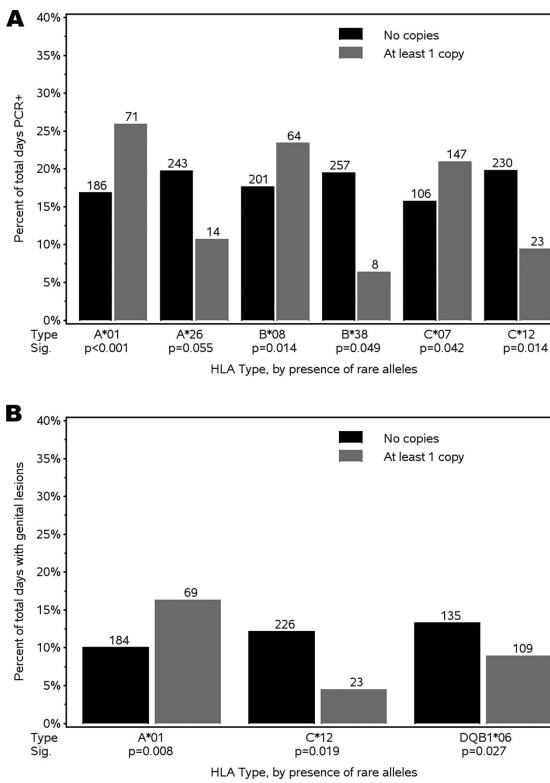
Figure 2.
a: HSV shedding frequency by HLA genes found to be significantly associated in univariate analysis. b: Genital lesion frequency by HLA genes found to be significantly associated in univariate analysis. "Sig." for "significance".
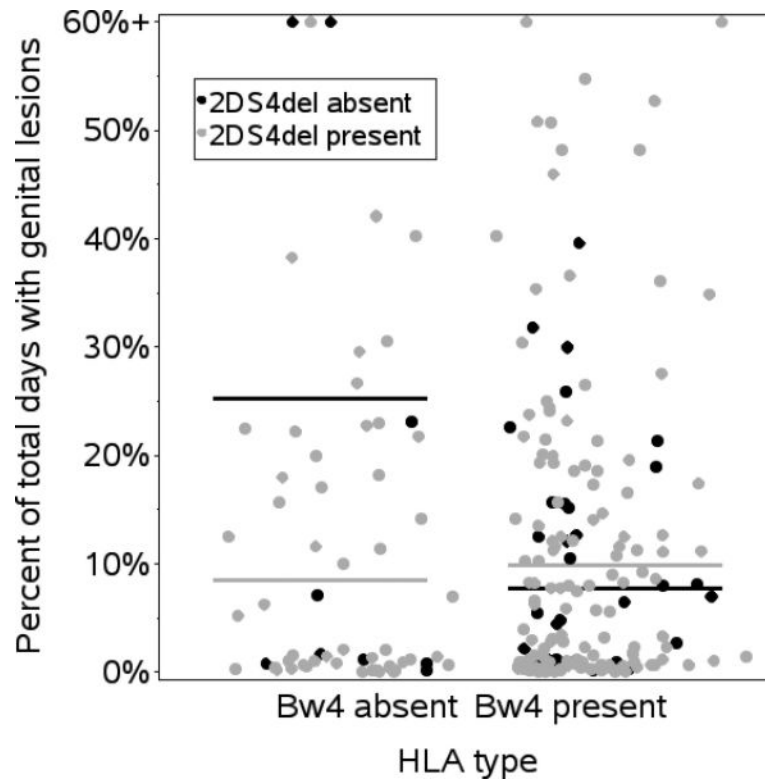
Figure 3.
Interaction effect between HLA-Bw4 and KIR-2DS4del allele for HSV genital lesion frequency. Horizontal bars indicate Poisson regression predicted lesion frequencies for each group.
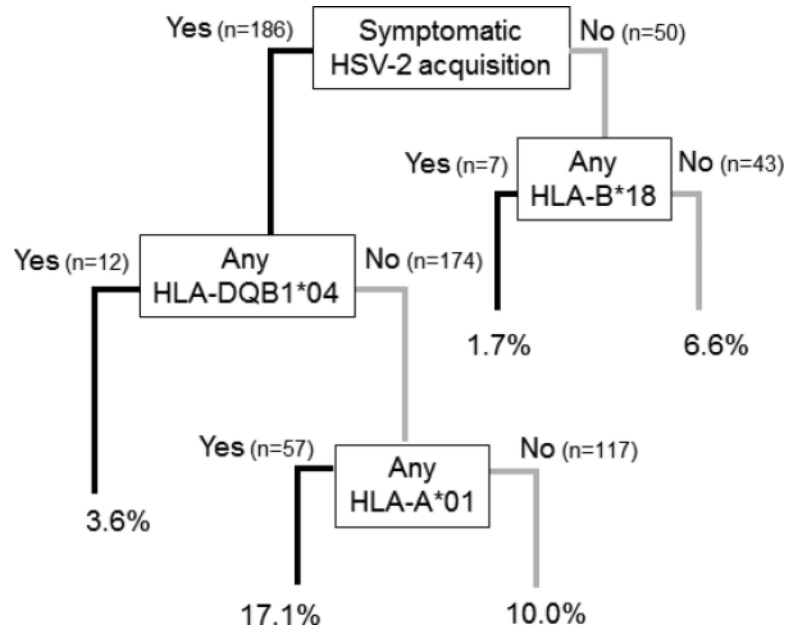
Figure 4.
Classification and regression tree results for HSV shedding rate, assessing its association with HLA types, gender, having a history of symptoms at the time of diagnosis, and HSV-1 coinfection. The HSV shedding rate within each category is indicated at the bottom of each branch.

Table 1

Demographic and clinical characteristics of study participants.

	Participants (n=267)
Age (median, range)	41 (20-76)
Women	161 (60%)
Serostatus	
HSV-2 antibody only	155 (58%)
HSV-1 and HSV-2 antibody	112 (42%)
Time since first episode of genital herpes	
Less than 1 year	36 (13%)
More than 1 year	174 (65%)
Unknown (asymptomatic acquisition)	57 (21%)
Total genital swabs contributed	16,440
Genital swabs contributed per person (median, range)	59 (30-168)
Swabs positive for HSV DNA	3,141 (19.1%)
Total diary days contributed	18,356
Diary days contributed per person (median, range)	62 (30-208)
Days with genital lesions	2,128 (11.6%)

Table 2a

HLA alleles found in at least 8 persons and evaluated for associations with HSV severity.

Persons with allele present									
HLA type	**n**	**HLA type**	**n**	**HLA type**	**n**	**HLA type**	**n**	**HLA type**	**n**
A*01	71	**B*07**	71	**C*01**	20	**DQB1*02**	98	**DRB1*01**	52
A*02	131	**B*08**	64	**C*02**	17	**DQB1*03**	142	**DRB1*03**	65
A*03	79	**B*13**	14	**C*03**	59	**DQB1*04**	18	**DRB1*04**	79
A*11	30	**B*14**	23	**C*04**	43	**DQB1*05**	63	**DRB1*07**	53
A*24	46	**B*15**	31	**C*05**	54	**DQB1*06**	110	**DRB1*08**	16
A*25	10	**B*18**	28	**C*06**	38			**DRB1*11**	43
A*26	14	**B*27**	27	**C*07**	147			**DRB1*12**	10
A*29	16	**B*35**	37	**C*08**	21			**DRB1*13**	54
A*30	10	**B*38**	8	**C*12**	23			**DRB1*14**	8
A*31	18	**B*40**	27	**C*14**	8			**DRB1*15**	68
A*32	12	**B*44**	73	**C*15**	13				
A*68	19	**B*49**	9	**C*16**	15				
A*68	71	**B*51**	27						
		B*57	13						

Table 2b

Genotype groups found in at least 8 persons and evaluated for associations with HSV severity.

Genotype	Persons with allele present
A3 supertype[a]	98
C group 1[b]	222
C group 2[c]	149
Bw4[d]	184
8.1 AH[e]	24
Cw4[f]	38

[a] either HLA-A*03 or A*11

[b] any HLA-C*01, *03, *07, *08, *12, *14, *16 as described by Khakoo and Chazara (55, 56)

[c] any HLA-C*02, *04, *05, *06, *15, *17, *18 as described by Khakoo and Chazara (55, 56)

[d] any number of copies of HLA-A*23, A*24, A*25, A*32, B*13, B*1513, B*1516, B*1517, B*1524, B*2702, B*2705, B*2709, B*3701, B*3801, B*3802, B*44, B*4701, B*4901, B*51, B*5201, B*5301, B*5302, B*57, B*58, by Martin (54)

[e] 8.1 ancestral haplotype (HLA-A*0101, B*0801, DRB1*0301, DQB1*0201) described in Price et al (5)

[f] any copies of HLA-C*0401

Table 3

Significance levels, prior to multiple comparison adjustment, for testing whether KIR alleles modify the influence of HLA type on genital HSV shedding rates and genital lesion rates.

HLA group[a]	KIR alleles							
	2DS1	2DL2	2DL3	2DS2	3DS1	3DL1	2DS4del	2DS4ins
C gp 1	0.99 / 0.38[b]	NA	NA	0.56 / 0.12	**0.0086** / 0.087	NA	0.15 / 0.084	0.73 / 0.86
C gp 2	0.32 / 0.84	0.30 / 0.48	0.43 / 0.44	0.31 / 0.63	0.89 / **0.027**	0.62 / 0.88	0.12 / **0.026**	0.50 / 0.58
Bw4	0.82 / 0.55	0.66 / 0.77	0.15 / 0.36	0.74 / 0.70	0.58 / 0.18	NA	0.24 / **0.0016**	0.77 / 0.34
A3/A11	0.28 / 0.34	**0.037** / 0.60	0.63 / 0.79	0.56 / 0.84	0.81 / 0.70	NA	0.50 / 0.40	0.73 / 0.62
Cw4	0.84 / 0.88	NA	NA	0.89 / 0.14	0.35 / 0.38	NA	0.14 / 0.20	0.16 / 0.26
CEH 2	0.85 / 0.91	0.32 / 0.46	NA	0.51 / 0.85	0.93 / 0.94	NA	NA	0.58 / 0.35

[c] Combinations of HLA and KIR types for which there were not a sufficient number of persons to analyze are indicated by "NA".

[a] HLA groups as per Table 2.

[b] p-values for interactive impact of the presence of both genetic markers on genital HSV shedding rates and genital lesion rates, separated by "/". p-values < 0.05 are bold and p-values remaining <0.05 after multiplicity adjustment are enclosed in a box.