HLA Immunogenotype Determines Persistent Human Papillomavirus Virus Infection in HIV-Infected Patients Receiving Antiretroviral Treatment

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Abstract

A proportion of human immunodeficiency virus (HIV)–infected patients develop persistent, stigmatizing human papillomavirus (HPV)–related cutaneous and genital warts and anogenital (pre)cancer. This is the first study to investigate immunogenetic variations that might account for HPV susceptibility and the largest to date to categorize the HPV types associated with cutaneous warts in HIV-positive patients. The HLA class I and II allele distribution was analyzed in 49 antiretroviral (ART)–treated HIV-positive patients with persistent warts, 42 noninfected controls, and 46 HIV-positive controls. The allele HLA-B*44 was more frequently identified in HIV-positive patients with warts (P = .004); a susceptible haplotype (HLA-B*44, HLA-C*05; P = .001) and protective genes (HLA-DQB1*06; P = .03) may also contribute. Cutaneous wart biopsy specimens from HIV-positive patients harbored common wart types HPV27/57, the unusual wart type HPV7, and an excess of Betapapillomavirus types (P = .002), compared with wart specimens from noninfected controls. These findings suggest that HLA testing might assist in stratifying those patients in whom vaccination should be recommended.

Introduction

Human immunodeficiency virus (HIV)–infected individuals are commonly afflicted with troublesome cutaneous and genital warts and genital precancer and cancer despite receipt of fully suppressive antiretroviral therapy (ART). The virus that causes warts, human papillomavirus (HPV), is classified on the basis of its DNA sequence into 5 genera, containing >120 different genotypes: Alphapapillomavirus (predominantly comprising genital HPV types but also including cutaneous wart types), Mupapillomavirus, Nupapillomavirus, Gammapapillomavirus (containing cutaneous wart types), and Betapapillomavirus (largely nonpathogenic cutaneous types) [1, 2]. HPV is ubiquitous; HPV types belonging to the Betapapillomavirus genus are detected with a high prevalence (>95%) in the general population, and it is estimated that around 75% of all individuals have been exposed to genital HPV types, mostly subclinically and without morbidity [3, 4]. HPV-related disease in HIV-infected patients is common and frequently results in a disabling and stigmatizing dermatosis, consuming significant clinical resources. HIV infection probably increases the prevalence of warts and other HPV-related diseases, and these diseases do not appear to abate with fully suppressive ART [5–8]. Persistent HPV genital disease in HIV-infected patients can lead to the development of precancerous lesions with the risk of progression to frank cancer. However, many individuals remain disease-free

despite presumed HPV exposure, whereas others are particularly troubled by HPV-associated diseases. This transition is likely to be augmented by long-standing HIV infection [9, 10]. The pattern and severity of skin disease in HIV-infected patients may be determined by factors that transcend their level of immunodysfunction, such as immunogenotype, of which $CD4^+$ T-cell count is just one marker. We investigated the immunogenotype (specifically, the HLA type) of HIV-infected patients with persistent viral warts and identified the HPV types in HIV-positive individuals with cutaneous and genital warts.

Methods

Subjects

Ethics approval was sought and received from the Riverside NHS Research Ethics Committee (London, United Kingdom) before study commencement. Informed written consent was taken from all patients. Participants were white Caucasoid (ie, they reported parental and grandparental ancestry from Northern Europe, defined as the United Kingdom, Ireland, Netherlands, Belgium, France, Germany, Switzerland, and the Scandinavian countries). In brief, 3 patient groups were recruited: (1) HIV-positive cases, defined as HIV-positive individuals with HPV disease and a history of persistent (duration, ≥6 months) cutaneous/genital warts (with or without a history of HPV related anogenital precancer); (2) HIV-positive controls, defined as HIV-positive patients without a history of persistent HPV disease; and (3) HIV-negative cases, defined as individuals without HIV infection and with HPV disease (as defined above).

All HIV-positive individuals were male, were infected with HIV-1 and had been taking fully suppressive ART for ≥1 year (HIV load <50 copies/mL for ≥6 months). HIV-positive controls had an absent or transitory past history (<3 months) of warts and no evidence of other HPV-related diseases, based on their medical history, case note review, and dermatological examination findings. Females with cutaneous warts (with or without genital warts) were included in the HIV-negative case group, but females with a history of genital warts or cervical abnormalities alone were not. HIV-negative cases were individuals with persistent warts in good physical health with no clinical indicators and no other risk factors for HIV infection (as assessed by private interview and thorough dermatological examination) and/or a negative result of an HIV antibody test. To be included as HIV-negative cases, men who have sex with men (MSM) either had a recent negative result of an HIV test or consented to HIV antibody testing (all test results were negative for HIV antibody). From a cohort of approximately 5000 HIV-infected patients attending the HIV clinic at Chelsea and Westminster Hospital (London) and from other clinics in the same hospital, 137 Caucasoid individuals were recruited (49 HIV-positive cases, 42 HIV-negative cases, and 46 HIV-positive controls). These individuals were HLA typed. A subset of HLA-typed

patients with warts at the time of review provided fresh wart tissue for HPV typing, resulting in 106 wart specimens from 30 HIV-positive cases and 36 HIV-negative cases. Demographic information was collected for all patients and included ethnicity, age, sex, total wart duration for cases (volunteered by the patient and corroborated by patient case records), year of HIV infection diagnosis and ART commencement, nadir CD4$^+$ T-cell count (corroborated by the year of ART initiation and defined as the lowest historical recorded CD4$^+$ T-cell count).

HLA Typing

DNA was extracted from fresh frozen whole-blood specimens, using standard Qiagen protocols (Qiagen, Crawley, United Kingdom) [11] and underwent HLA typing by a validated laboratory (Anthony Nolan Histocompatibility Laboratories, London), using a commercial Luminex-based platform (One Lambda LABtype SSO typing, VH Bio, Gateshead, United Kingdom). HLA allele frequencies for Caucasoid individuals derived from UK based populations were also available [12–14]. HLA-A, HLA-B, and HLA-DRB1 allele frequencies were taken from a population of 5024 Caucasoid blood donors who attended a UK blood transfusion centre; HLA-C frequencies were derived from a subgroup of 2420 individuals from the same population. HLA-DQB1 frequencies were from 177 individuals included in a London anthropology study comprising subjects from England, Scotland, Wales, and Northern Ireland; HLA-B*44 allele subtypes were obtained from 298 Caucasoid blood donors from Liverpool, United Kingdom.

HPV Typing

Study subjects with warts were offered surgical treatment (curettage/shave biopsy or excision). Wart biopsy specimens were divided: half were sent in 10% formalin for confirmatory histopathologic analysis, and the remaining fresh tissue specimens were snap frozen and used for DNA extraction; one of the 106 samples was derived from recently processed formalin-fixed paraffin-embedded tissue. Clinically diagnosed (by a dermatologist) viral warts that were located on sites not easily amenable to surgical biopsy under local anesthetic (eg, plantar, periungual, and finger warts) were pared deeply with a scalpel, and the parings were used for HPV typing. DNA was extracted from tissue by using standard Qiagen protocols as described above [11], with the addition of the following stringent anti-contamination procedures: extended tissue lysis, a longer elution time, and a smaller elution volume. DNA from cutaneous wart associated HPV types was detected using 2 techniques: (1) nested PCR/sequencing designed to detect HPV types 3, 10, 28, 29, 77, 2, 27, 57, 1, 41, and 63, as well as other potentially novel types [15]; and (2) a newly developed Luminex-based platform (HSL-PCR/MPG), using broad-spectrum primers and probes to detect both the types specified above and the additional HPV types 7, 4, 40, 43, 48, 50, 60, 65, 88, 91, 94, and 95 [16]. Thus, 23 cutaneous wart associated HPV types were detectable. Genital and

Betapapillomavirus types were distinguished using 2 separate commercial reverse hybridization line probe assays. The genital assay [17, 18] identified 25 HPV types: high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 70 and low risk HPV types 6, 11, 34, 40, 42–44, 53, 54, and 74 (HPV SPF_{10}-DEIA-LiPA$_{25}$ system, version 1 [based on licensed Innogenetics technology]; Labo Biomedical Products, Rijswijk, the Netherlands). The Betapapillomavirus assay [19] contained probes that detected 25 HPV types: HPV 5, 8, 9, 12, 14, 15, 17, 19, 20, 21, 22, 23, 24, 25, 36, 37, 38, 47, 49, 75, 76, 80, 92, 93, and 96 (Diassay B.V., Rijswijk, The Netherlands).

Statistical Analysis

Descriptive and analytical statistics for HPV data were computed with the use of the following standard software programs and with the advice of the Statistical Advisory Service and St Stephens Centre (Imperial College, London): Microsoft Excel; Statistical Package for the Social Sciences (SPSS), version 20; and Epi Info (Centers for Disease Control and Prevention; available at: http://www.cdc.gov/epiinfo/; accessed 20 January 2009). Nonparametric data were analyzed with the Mann– Whitney U test. Categorical data were analyzed with Yates' corrected χ^2 or Fisher exact tests (2-tailed). Advice regarding HLA allele counting and immunophenotypic analysis was provided by the Anthony Nolan Histocompatibility Laboratories. A substantial Bonferroni correction for multiple testing was applied to each HLA analysis, to account for all potential allele comparisons, and was accomplished by multiplying the calculated P value by 135 (denoted "$P_{corrected}$").

Results

Patient Attributes

Results of HLA typing of DNA from blood specimens were available for 49 HIV-positive cases and 42 HIV-negative cases with persistent warts (Table 1). Of these, most (86% [42 of 49] and 69% [29 of 42], respectively) had a history of cutaneous warts; 39% (19 of 49) and 45% (19 of 42), respectively, had a history of genital warts, and 24% (12 of 49) and 14% (6 of 42), respectively, had a history of both. Three individuals among HIV-positive cases and 3 among HIV-negative cases also had a history of HPV-related penile or anal cancer in situ. The median duration of self-reported warts in this study was between 3 and 5 years for both HIV-positive cases and HIV-negative cases; this long duration is expected because individuals with persistent warts for six months were recruited. HIV-positive cases and HIV-positive controls included significantly more males (100%; P < .0001) and MSM (98%; P < .0001) than HIV-negative cases, a function of the demographic characteristics of HIV clinic attendees (largely male); among HIV-

negative cases, 29% (12 of 42) were female and 15% (6 of 42) were MSM. Females with cutaneous warts were also included as HIV-negative cases because cutaneous warts in males and females are probably comparable. There were no other significant differences between study subjects.

Molecular HLA data were reported as a standard string consisting of 2 allele fields (as defined by the assay manufacturer). The first field was used to determine the allele distribution, by phenotypic allele counting. Therefore, a homozygous individual counted only once toward the allele total (Figures 1 and 2 and Supplementary Table 1).

The main HLA allele of interest that emerged from this study was HLA-B*44 allele: 47% of HIV-positive cases (23 of 49) with HPV disease had this allele, compared with 17% of HIV-positive controls (8 of 46; P = .004; $P_{corrected}$ = .54) and with 32% of the blood donor population (1608 of 5024; P = .04; $P_{corrected}$ = 1.0; Figure 1). The HLA-B*44 allele count for HIV-negative cases was between that for HIV-positive cases and HIV-positive controls, at 33% (14 of 42). Further interpretation of the allele subgroups revealed that HLA-B*44:02 was the predominant HLA-B*44 subtype; it was present in 38% of HIV-positive cases (18 of 47), 7% of HIV-positive controls (3 of 46; P = .0006; $P_{corrected}$ = .04), and 19% of individuals (57 of 298) from a comparative United Kingdom Caucasoid blood donor population (P = .006; $P_{corrected}$ = .43). Another allele, HLA-C*05 was also seen more frequently in HIV-positive cases (33% [16 of 49]), compared with HIV-positive controls (9% [4 of 46]; P = .009; $P_{corrected}$ = .59). HLA-B*44 and HLA-C*05 are in linkage disequilibrium and constitute a common Caucasoid haplotype. Thirty-seven percent of HIV-positive cases (18 of 49) had the haplotype HLA-B*44, -C*05, compared with only 7% of HIV-positive controls (3 of 46; P = .001; $P_{corrected}$ = .07). The allele HLA-DQB1*06 may also be of possible of interest because it was detected more frequently in HIV-positive controls (which makes it potentially protective against HPV disease), compared with United Kingdom Caucasoid blood donors (61% [28 of 46] vs 42% [74 of 177]; P = .03; $P_{corrected}$ = .8). There was no significant difference between the allele frequency of HLA-DQB1*06 among HIV-positive cases (43% [21 of 49]) and that among HIV-positive controls (61% [28 of 46]; P = .12).

There was no significant difference in the number of subjects from each study group who were homozygous for least 1 allele at HLA-A, -B, -C, -DRB1, and -DRB1 loci: 41% of HIV-positive cases (20 of 49) were homozygotes, compared with 43% of HIV-positive controls (20 of 46) and 57% of HIV-negative cases (24 of 42; P = .24).

HPV

The characteristics of individuals in the HIV-negative and HIV-positive case groups who contributed HPV typing data from wart tissue specimens were almost identical to those of individuals included in the HLA analysis. HPV typing was performed for 30 HIV-positive cases (using tissue specimens from 38 cutaneous warts and 10 genital warts) and 36 HIV-negative cases (using tissue specimens from 46 cutaneous warts and 12 genital warts). All 106 sampled warts were positive for at least 1 HPV type (Figures 3 and 4 and Supplementary Table 2). However, in specimens from 4% of cutaneous warts (3 of 84) and 5% of genital warts (1 of 22), no likely pathogenic HPV type was identified, although Betapapillomavirus types were detected at low levels.

Mixed HPV infections were common. For the cutaneous wart associated HPV types and genital HPV types combined, two or more HPV types from either group were detected in 29% (31 of 106) of warts. For the ubiquitous Betapapillomavirus HPV types, the detection of multiple types was higher in HIV-positive cases than in HIV-negative cases (60% [29 of 48] vs 38% [22 of 58]; P = .03). The median number of Betapapillomavirus types was also greater in HIV-positive cases than in HIV-negative cases (3 [interquartile range {IQR}, 0.3–8] vs 1 [IQR, 0–2]; P = .002). HPV 27 and 57 were the most commonly identified HPV types in all cutaneous warts (from both HIV-positive and HIV-negative cases; Figure 3). HPV 2 was more frequently detected in specimens of cutaneous warts from HIV-negative cases than those from HIV-positive cases (30% [14 of 46] vs 3% [1 of 38]; P = .002). HPV 7 was identified in specimens of 8% of cutaneous warts (4 of 38) from HIV-positive cases and in 0% (0 of 46) from HIV-negative individuals (P = .04). The HPV 7–positive warts were located on the face and head. After correction for multiple warts from a single individual, this difference for HPV 7 was not statistically significant, with detection in 3 of 27 HIV-positive cases and 0 of 26 HIV-negative cases (P = .2). HPV 11 and HPV 6 were identified in genital wart specimens from both HIV-positive and HIV-negative cases, and there was no difference in the HPV type prevalence; the number of genital warts examined was small.

Discussion

To our knowledge, this work is the first to examine the immunogenotypic profile of HIV-infected individuals with warts. The HLA class I allele groups HLA-B*44 (as the allele subtype HLA-B*44:02) and HLA-C*05 were more frequent in HIV-positive cases with persistent warts than in HIV-positive controls. The class II allele HLA-DQB1*06 was found more often in HIV-positive controls than in HIV-positive cases, implying it might have a moderate, possibly protective effect. However, after a conservative Bonferroni correction (ie, multiplication of the P value by 135), none of these associations were statistically significant, except for that of HLA-B*44:02 ($P_{corrected}$ = .04).

The differences in the allele distributions suggest an HLA class I predisposition to HPV disease in HIV-infected individuals. The potential mechanisms behind HLA associations with HPV disease in individuals with treated HIV infection might operate at several levels; 2 viral pathogens are involved.

The best evidence to date for HPV diseases (ie, data on cervical cancer and recurrent respiratory papillomatosis) suggests that predominantly HLA class II alleles appear to govern the host response to HPV. HLA-DRB1*13:01/02 and -DQB1*06:03 have been associated with protection against HPV diseases, whereas HLA-DQB1*06:02 has been identified with discordant associations (protective, neutral, or deleterious) [20–22]. Other alleles, such as HLA-DRB1*03:01 and HLA-DQB1*03:01/02/03, and the haplotype HLA-DRB1*15:01, -DQB1*06:02 have been found to confer susceptibility [21, 23, 24]. There are few reports describing class I associations in HPV. However, HLA-B*44 was previously correlated, in a small number of subjects, with increased progression of HPV 16–related cervical dysplasia [21, 25]. More data are provided by 2 studies that examined HLA class II (but not HLA class I) in immunocompetent individuals with cutaneous warts. None, to our knowledge, have studied HIV-infected patients. HLA-DRB1*03 and HLA-DRB1*09 were found more frequently in Mexican individuals with cutaneous warts than in the general population, and HLA-DR6 (HLA-DRB1*13/14)

was found to be protective [26]. A German study compared 71 otherwise healthy individuals with persistent cutaneous warts lasting >18 months that were positive for HPV 2, 27, and 57 to 92 controls [27]. A statistically significant increase was seen in HLA-DQB1*03:01 and HLA-DQA1*03 in cases, compared with controls, consistent with previous studies in cervical cancer; nonsignificant trends were also identified for alleles, including HLA-DQB1*06:03 (protective). The data from the investigation herein in HIV-infected individuals suggest that HLA-DQB1*06 (all alleles combined) might be protective against HPV. Others have suggested this same allele may be protective in other HPV-related diseases, such as cervical cancer/precancer and recurrent respiratory papillomatosis [21]. One HPV-protective HLA class II allele may be insufficient to prevent HPV disease in HIV-infected individuals, and other protective alleles and/or fewer predisposing alleles may be necessary, such as the haplotype HLA-B*44, -C*05 in the absence of -DQB1 that was seen in some of our HIV-positive cases.

HIV disease progression, on the other hand, is thought to be HLA class I related. Alleles protective for HIV may be able to generate an effective CD8+ T-cell response to highly conserved HIV p24 peptides, while deleterious alleles may direct an ineffective immune response [28, 29]. HLA-B*27 and -B*57 have historically been most consistently associated with delayed HIV progression, and HLA-B*35 has been associated with HIV disease that progresses more rapidly.

Other work suggested that the HLA-Bw4 group of alleles (HLA-B*44 is one) may influence the function of natural killer receptors (KIRs) in HIV infection [30, 31]. However, alleles such as HLA-B*27:02, HLA-B*57 (rather than HLA-B*44) are preferential ligands for the KIRs KIR3DL1/KIRDS1, and therefore the effect of weaker ligands such as HLA-B*44 may be clinically insignificant. It is possible that the allele of interest, HLA-B*44:02, is not related to HPV disease and may be a marker of greater HIV disease progression that is not reflected in variables such as HIV infection duration or nadir CD4$^+$ T-cell count. Additionally, class II HLA molecules might affect thymic T-cell education. The thymus may be of particular importance in HIV infection during ART receipt because of the recognized emergence of naive CD4$^+$ T-cells during immune recovery; this ability declines with aging, as does HIV-related prognosis [32].

This study is also the first to comprehensively categorise the HPV type profile of warts from HIV-infected patients. It supports the limited evidence to date (from case reports) that HPV 7 may be an important pathogen in HIV-infected patients [33–35] and that HPV types commonly associated with warts (such as HPV 27/57 in cutaneous warts) were frequently detected in wart specimens from HIV-infected patients. The only other study that typed HPV from a substantial collection of warts from HIV-infected patients found HPV 7 in specimens from 4% of wart specimens (1 of 25) in HIV-infected individuals but not in 14 wart specimens from non–HIV-infected subjects [36]. In the general population, HPV 7 has been reported infrequently (in 0.5%–2% of individuals) and in unusual sites [16, 37–43]. Immunodysfunction may be important; HPV 7 was found in 17% of 23 wart specimens from 12 organ transplant recipients but was absent in several other studies that typed HPV in wart specimens from immunosuppressed patients [15, 44–47]. However, it is possible that rarer HPV types (such as HPV 7) may be overrepresented in our HIV-infected cohort owing to propagation of the infection between individuals in close contact within the cohort (ie, the cohort is epidemiologically closed). MSM may potentially carry more HPV types, particularly genital types, through greater exposure to HPV. HPV 1 has been reported to be a common HPV type in cutaneous warts [38] but was absent from both HIV-positive cases and HIV-negative cases in this study. A predilection of HPV 1 for children, plantar warts, and warts of short duration may have excluded HPV 1 from our study [37, 40, 43]. The excess of HPV 2 in warts from HIV-negative cases might be a compensatory phenomenon related to the smaller number of HIV-negative patients that harbor HPV 57.

What was also apparent from these results is the greater median number of Betapapillomavirus types in warts from HIV-infected patients and the higher proportion of warts with mixed Betapapillomavirus types. This excess of Betapapilloma-virus types in HIV-infected patients might be related to immunodysfunction (similar to the situation in organ transplant recipients) or due

to the number of close contacts or lifetime sex partners (as appears to be the case for genital HPVs).

Epidermodysplasia verruciformis–like eruptions have been reported in HIV-infected individuals [48], although none of the individuals included in this study had this clinical phenotype.

In summary, HIV-infected individuals with warts were more likely to be HLA-B*44 positive and have the haplotype HLA-B*44, -C*05 than either HIV-infected controls or the general population. The potentially protective allele HLA-DQB1*06 was increased in HIV-positive controls to lesser degree. However, the power of the study is too small to draw definite conclusions, and further work is necessary to validate our findings. We have also characterized the HPV profile in this same group of Caucasoid patients and identified some differences from a control population; in particular, our data suggest that HPV 7 may be an important pathogen in HIV-infected patients.

The emergent alleles of interest fit within the context of previous work and potentially may involve the interaction of HLA and KIR ligands. Immunogenotypic influences are unlikely to compose the entire picture, and other factors probably are important in HPV disease during treated HIV infection, such as as-yet unelucidated or unquantified immunodysfunction or variations in immune adaptivity. These might include thymic generation of naive $CD4^+$ T cells, immunosenescence or host phenotypic factors such as $CD4^+$ T-helper cell type 1/2 polarization, HIV-associated $CD4^+$ T-cell predominant immune decimation and dysregulation, regulatory T-cell function, natural killer cells, and/or $CD8^+$ T-cell immune responses.

Regarding the clinical usefulness of our HLA findings, they are consistent with the presence of immunosusceptible and immunoprotective genotype(s). This adds indirectly to the argument and rationale for prophylactic HPV vaccination in boys, as well as for postexposure HPV vaccination in the management of morbidity and diminution of mortality due to HPV-related disease in men (and women) in all settings. Although prophylactic vaccines may not be therapeutic, vaccination can impart cross-reactive immunity to other HPV types [49]. This is most beneficial among younger individuals (age, <26 years); MSM; persons at higher risk of HPV-related morbidity, such as HIV-infected patients [50], and perhaps those who immunogenetically predisposed to HPV infection. Our clinical practice is increasingly to recommend HPV vaccination in these settings.

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	HIV+ cases	HIV+ controls	Non-Infected cases	HIV+ cases compared with HIV+ controls p=significance level	HIV+ cases compared with NI cases p=significance level
Table 1: Study Participant Demographics Group					
Number of individuals (n)	49	46	42	—	—
Age years median (IQR)	45 (41-54)	49.5 (44-56)	41 (32-52)	p=0.05	p=0.06
Sex (% male)	100%	100%	71%	p=1*	p<0.0001*
HIV Infection years median (IQR)	14 (8-19)	11 (7-20)	—	p=0.62	—
ART years median (IQR)	9.0 (5-13)	8.5 (5-12)	—	p=0.96	—
Risk factors for HIV (% MSM)	98%	98%	15%	p=1*	p<0.0001*
Current CD4 T-cell cells/µl median (IQR)	483 (343-693)	544 (431-683)	—	p=0.41	—
CD4 T-cell nadir cells/µl median (IQR)	148 (41-205)	137 (48-205)	—	p=0.86	—
Viral load % undetectable (<50 copies/ml)	100%	100%		p=1*	—
Wart disease years median (IQR)	4.0 (1.8-9)	—	3.5 (1.4-5)	—	p=0.42

Figure 1: HLA-B allele count distribution as percentage of individuals in each group
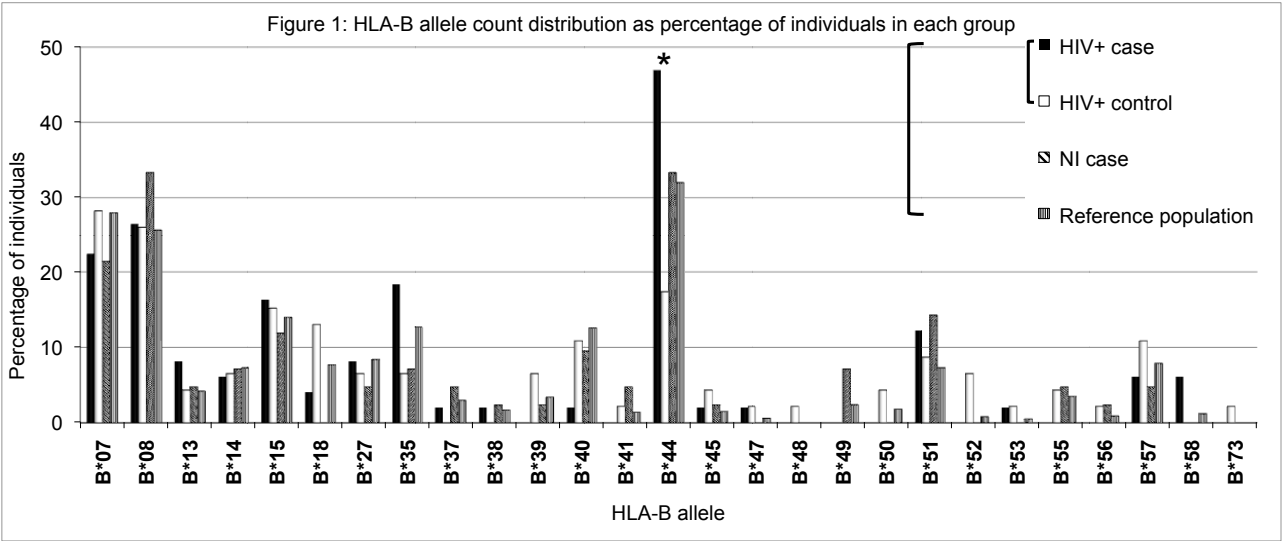
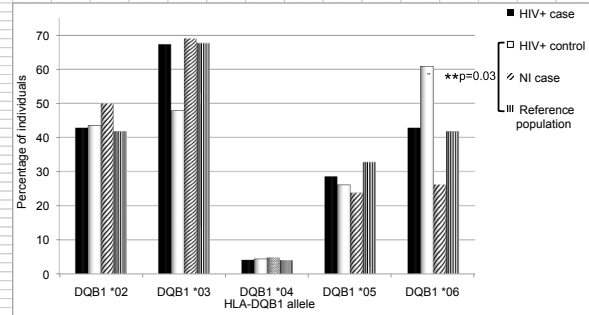Figure 2: HLA-A, HLA-C, HLA-DRB1 and HLA-DQB1 allele count distributions as percentage of individuals in each group
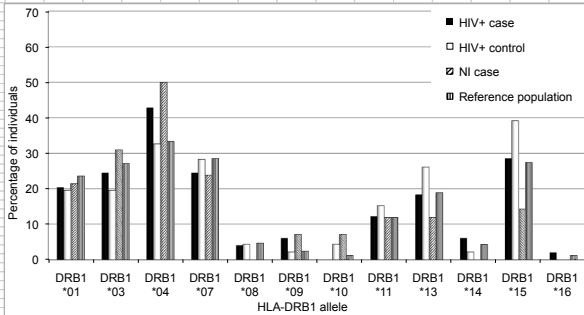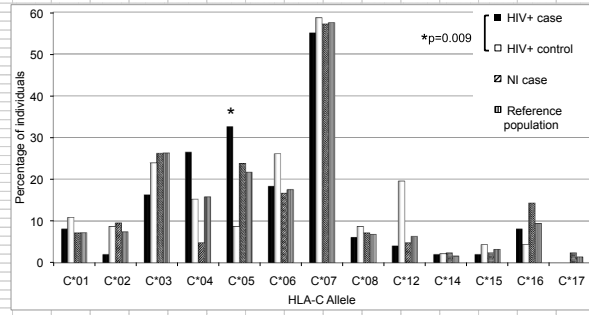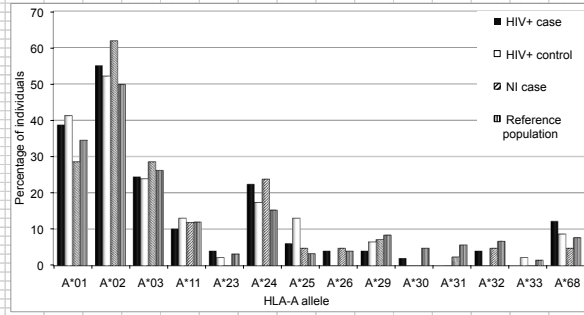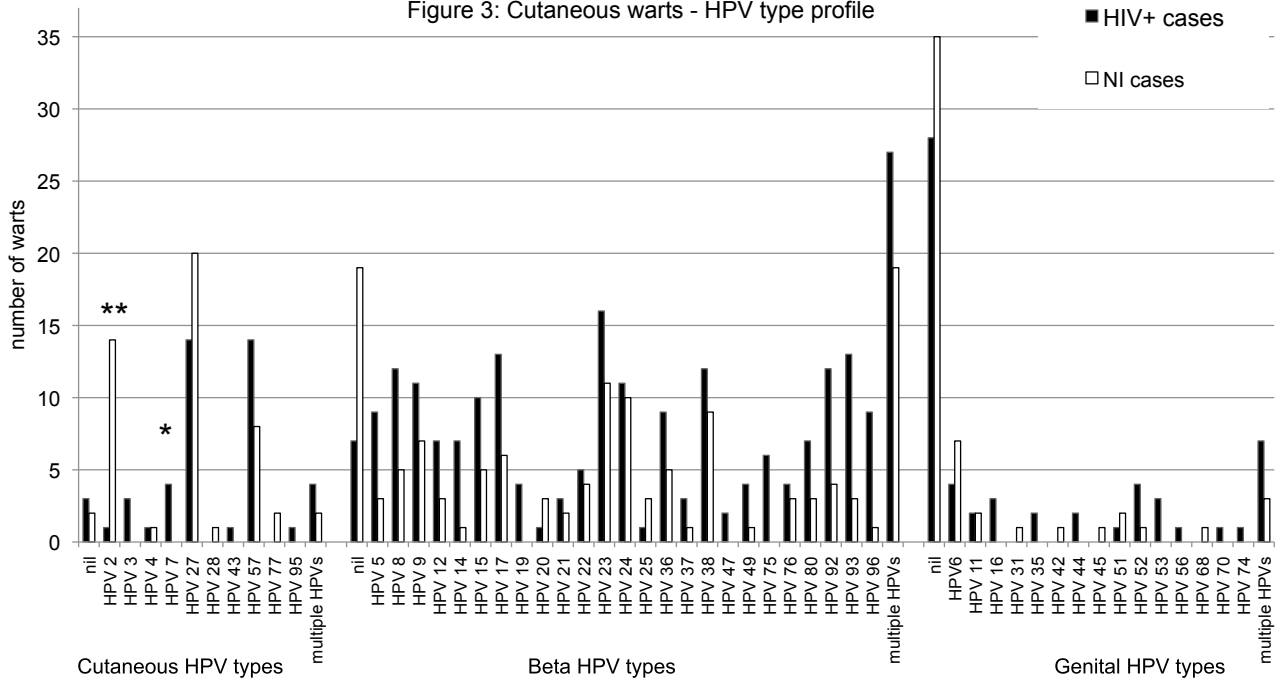
Figure 3: Cutaneous warts - HPV type profile

Figure 4: Genital warts - HPV type profile