

Review

Host and viral genetics and risk of cervical cancer: a review

Allan Hildesheim*, Sophia S. Wang

Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Blvd, Room 7062, EPS/MS# 7234, Rockville, MD 20852, USA

Abstract

Infection with human papillomaviruses (HPV) is known to play a central role in the development of cervical cancer. Both host and viral genetic factors have been postulated to be important determinants of risk of HPV progression to neoplasia among infected individuals. In this report, we review epidemiological studies that have evaluated the role in cervical cancer pathogenesis of genetic variation in human leukocyte antigen (HLA) genes and in the HPV genome itself. A protective effect of HLA Class II DRB1*13/DBQ1*0603 alleles is the most consistent HLA finding in the published literature. A consistent association between HPV16 non-European variants and risk of disease is also evident from published work. These findings are discussed. Gaps in our understanding and future research needs are also discussed.

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1. Introduction

Having demonstrated that infection with one of approximately two dozen oncogenic human papillomavirus (HPV) types is necessary for the development of cervical cancer, the new challenge for the HPV/cervix cancer research community is to define additional factors that are involved in the persistence and progression of HPV infections to cervical cancer (IARC, 1995). Because only a small fraction of HPV infected women ever develop cervical cancer, these additional risk factors are

likely to be important determinants of disease risk. Along with environmental and lifestyle factors, host and viral genetic factors are likely to play a role in this process. It is recognized that host genetic factors linked to the development of cervical neoplasia are likely multigenic. However, in the present report we focus on the review of epidemiological evidence linking one set of host genetic factors, human leukocyte antigen (HLA) genes, and viral genetic variation to cervical cancer pathogenesis.

2. Human leukocyte antigens (HLA)

Herein we summarize epidemiological studies that have evaluated the association between spe-

* Corresponding author

E-mail addresses: hildesha@exchange.nih.gov (A. Hildesheim), wangso@mail.nih.gov (S.S. Wang).

cific HLA alleles/haplotypes and risk of cervical neoplasia. We will not review studies on the well established downregulation of HLA Class I molecules and upregulation of HLA Class II molecules in cervical neoplastic lesions; rather, we refer those interested in HLA expression studies to several recent publications on this topic (Coleman and Stanley, 1994; Chatterjee et al., 2001; Evans et al., 2001; Garrido et al., 1997; Koopman et al., 2000).

2.1. *Biological plausibility for an HLA effect*

There is strong a-priori biological plausibility supporting a role of HLA antigens in the development of HPV-related cervical cancer. Since HLA molecules are responsible for the presentation of foreign antigens to the immune system, they play a central role in the immune recognition and subsequent clearance of virally-infected cells (Paul, 1999). It is believed that possession of HLA molecules that bind HPV antigen with high affinity might be associated with protection against disease, while possession of HLA molecules that do not recognize and bind HPV antigens would be associated with increased risk of disease. Since a key feature of genes in the HLA complex is their high degree of polymorphism, and because genetic polymorphism within the HLA complex is a key determinant in affinity of peptide binding, the potential for identifying individuals with differential risks for cervical cancer is believed plausible.

HLA comprises a family of genes within the major histocompatibility complex located on the short arm of chromosome 6 (6p) in humans. This family of genes is divided into two major classes, Class I and Class II genes. HLA Class I genes (most notably HLA A, B, and C genes) are constitutively expressed in nucleated cells and are known to be essential for targeting of infected cells for killing by cytotoxic T-cells (acquired immune response). HLA Class I molecules present foreign antigens to CD8⁺ cytotoxic T lymphocytes, and these CD8⁺ T cells kill cells that have foreign antigens presented to them by HLA Class I molecules. HLA Class I genes are also important in innate immune responses, since natural killer

cells, which are central to innate immune responses, rely on the presence of these antigens on the cell surface to distinguish self from non-self. Cells that lack self Class I antigens on their surface are often targeted for killing by natural killer cells. HLA Class II genes (most notably DR, DQ, and DP α and β genes) have a more restricted pattern of expression, being typically expressed in cells of the immune system (e.g. macrophages and lymphocytes), and are known to be important in the regulation of the immune response to viral and other infections. Class II molecules present antigenic peptides to CD4⁺ T helper cells to initiate a cell-mediated immune response. For additional information on HLA and its role in innate and acquired immune responses, we refer readers to other publications on this topic (Sell et al., 1996).

Class I and Class II HLA genes are highly polymorphic. This diversity is favored for evolutionary survival, since it enables resistance to potential pathogens by binding and presenting a wide variety of antigenic peptides. In addition, the multiple genes within the Class I and II regions further allow for variety in antigenic peptide presentation; for example, an individual heterozygous at all three Class I loci (A–C) would express six different Class I proteins. However, this high degree of polymorphism makes identifying associations between specific HLA alleles or haplotypes (haplotypes are combinations of HLA genes inherited together from one parent) and disease difficult due to the low prevalence in a given population of any particular allele or haplotype.

To date, most studies of HLA and cervical neoplasia have focused on HLA Class II genes. This research bias for HLA Class II genes was driven by the availability of robust HLA Class II genotyping assays rather than scientific considerations. PCR-based methods for the identification of HLA Class II genotypes (rather than the conventional serotypes that often group large numbers of individual genotypes into a single serogroup) were developed earlier than Class I genotyping methods, making Class II studies easier to perform than Class I studies. However, this is no longer the case.

2.2. Studies of HLA Class II genes and risk of cervical neoplasia

Many case-control studies of modest size have examined the association between HLA Class II (DR, DQ, DP) genes and cervical disease. As shown in [Table 1](#), populations studied include women from Europe, North America, Asia, Africa, and South and Central America, thus encompassing a large array of ethnic groups. Although initial studies measured HLA using serologic testing methods, the majority of studies report HLA Class II alleles typed using more specific genotyping techniques. Cases were mostly defined as women with cervical cancer or high-grade precursor lesions, with a few studies having the ability to examine the entire natural history spectrum of cervical disease from infection to high-grade disease and cancer. Comparable control populations remains a challenge, as few studies assessed controls by HPV status, and few case-control groups were derived from a clear population base. Nevertheless, these studies have individually and collectively examined a large number of HLA alleles over the past decade. Three groups of HLA alleles/haplotypes have been the most extensively studied and include (1) DQB1*03 alleles (DQB1*0301, DQB1*0302, and DQB1*0303); (2) DRB1*1501 and DQB1*0602 alleles (two alleles that are in linkage disequilibrium, and therefore, often occur on the same haplotype); and (3) DRB1*13 (consisting of DRB1*1301–5 alleles) and DQB1*0603 (DRB1*1301 and DQB1*0603 are also in linkage disequilibrium). The first two sets of alleles listed above have been hypothesized to be associated with increased disease risk while the last set is believed to be associated with decreased risk of disease. [Table 1](#) summarizes the results of studies published to date with respect to these three groups of HLA alleles.

The most consistent findings were for DRB1*13 and/or DQB1*0603, where evidence for protection was evident for 18 (95%) of 19 studies. In nine (47%) of these studies the effect was statistically significant. In US Hispanic and Costa Rican women possessing these alleles, significant odds ratios of 0.3–0.4 were reported for protection against cervical cancer ([Apple et al., 1994](#); [Wang](#)

[et al., 2001](#)). Similarly, studies of European Caucasians in UK, Sweden, and France reported significant odds ratios of 0.3–0.4 for precancer as well as cancer outcomes ([Odunsi et al., 1996](#); [Sastre et al., 1996](#); [Sanjeevi et al., 1996](#)). In some studies with the ability to assess HPV type-specificity, namely for HPV 16, the associations were more pronounced, although not uniformly so ([Apple et al., 1994](#); [Hildesheim et al., 1998](#)). Despite these consistent findings, at this point it is unclear which DRB1*13 allele(s) are important, whether DRB1*13 alleles alone, DQB1*0603 alone, or both are associated with reduced risk of disease, or whether other genes in the MHC complex found in linkage disequilibrium with these HLA alleles are important. Larger studies will be required to dissect out the independent effects of these alleles. Also, to further enhance our biological understanding of these effects, identification of specific viral epitopes presented by these protective alleles will be essential.

The evidence is less convincing for the risk alleles DQB1*0301–3 and DRB1*1501/DQB1*0602. Slightly more than half (59%) of the 27 studies conducted to date provided some support for increased risk of disease associated with one or more DQB1*03 alleles (11 [41%] of these studies provided statistically significant findings). While statistically significant increases in risk (approximately 2-fold) for cervical cancer and precancers were observed in European and US Caucasian women ([Sastre et al., 1996](#); [Sanjeevi et al., 1996](#); [Wank and Thomssen, 1991](#); [Helland et al., 1992](#); [David et al., 1992](#); [Helland et al., 1994](#); [Odunsi et al., 1995](#); [Cuzick et al., 2000](#); [Neuman et al., 2000](#)), African–American women ([Gregoire et al., 1994](#)), and Japanese women ([Nawa et al., 1995](#)), an equal number of null associations were also observed for women in Europe ([Glew et al., 1993](#); [Helland et al., 1998](#); [Krul et al., 2000](#); [Brady et al., 1999](#); [Beskow et al., 2001](#)), South and Central America ([Maciag et al., 2000](#); [Wang et al., 2001](#)), and Africa ([Lin et al., 2001](#)).

Nine (43%) of 21 studies provided support for an effect of DRB1*1501 and/or DQB1*0602 (seven [33%] statistically significant). An increased risk for cervical cancer associated with the DRB1*1501/DQB1*0602 alleles was first reported

Table 1
Summary of studies that have evaluated the association between HLA Class II alleles/haplotypes and cervical neoplasia

| Authors | Country | Typing method | Study groups | DQB1*0301–3 | DRB1*1501, DQB1*0602 | DRB1*1301–5, DQB1*0603 |
|----------------------------|-----------------------|---------------------|---|-----------------|----------------------|------------------------|
| Wank and Thomssen (1991) | Germany | Serology | 66 Cancers; 109 Controls; 2019 IWC ^a | ++ ^b | 0 ^b | -- ^b |
| Glew and Stern (1992) | UK | Serology | 64 Cancers; 857 Controls | – | 0 | – |
| Helland et al. (1992) | Norway | PCR LR ^c | 168 Cancers; 118 controls | ++ | ND ^d | ND |
| David et al. (1992) | UK | PCR LR | 30 CIN3; 49 controls; 50 blood donors | ++ | ND | ND |
| Vandenvelde et al. (1993) | Belgium | PCR LR | 26 CIN3/CIS; 45 CIN1/2; 323 controls; 288 blood donors | 0 | ND | ND |
| Glew et al. (1993) | UK | Serology and PCR LR | 57 Cancers; 857 controls | – | + | – |
| Apple et al. (1994) | USA, Hispanics | PCR | 98 Cancers; 220 controls | + | ++ | -- |
| Gregoire et al. (1994) | USA, African–American | PCR | 66 Cancers; 214 controls | ++ | 0 | ND |
| Mehal et al. (1994) | UK ^c | PCR LR | 28 Cancers; 24 CIN3/CIS; 15 CIN1; 73 controls | + | ND | ND |
| Helland et al. (1994) | Norway | PCR | 158 Cancers; 156 controls | ++ | 0 | ND |
| Nawa et al. (1995) | Japan | PCR LR | 23 Cancers; 307 IWC | ++ | ND | ND |
| Apple et al. (1995) | USA, Hispanics | PCR | 73 CIN3/CIS; 55 CIN1/2; 220 controls | -- | ++ | – |
| Odunsi et al. (1995, 1996) | UK | PCR | 176 CIN; 416 controls | ++ | 0 | -- |
| Satre-Garau (1996) | France | PCR | 126 Cancers; 165 controls | ++ | 0 | -- |
| Sanjeevi et al. (1996) | Sweden | PCR | 74 CIN1-3; 164 controls | ++ | ++ | -- |
| Hildesheim et al. (1998) | USA, Caucasians | PCR | 141 HSIL; 202 LSIL; 166 HPV+ Controls; 202 HPV-controls | + | -- | – |
| Helland et al. (1998) | Norway | PCR | 92 CIN2/3; 225 controls | 0 | ++ | – |
| Ferrera et al. (1999) | Honduras | PCR | 24 CIN3/Ca; 25 CIN1/2; 75 controls | + | – | – |
| Krul (1999) | Netherlands | Serology | 173 Cancers; 116 CIN; 1161 blood donors | 0 | ++ | -- |
| Montoya et al. (1998) | Spain | PCR | 34 Cancers; 78 CIN3/CIS; 30 CIN1/2; 138 controls | + | 0 | -- |
| Brady et al. (1999) | UK | PCR | 76 Cancers; 144 blood donors | 0 | 0 | 0 |
| Maciag et al. (2000) | Brazil | PCR | 161 Cancers; 257 controls | 0 | ++ | – |
| Cuzick et al. (2000) | Scotland, UK | Sequencing | 116 Cancers; 155 controls | ++ | + | – |
| Neuman et al. (2000) | US | Sequencing | 96 Cancers & Parents | ++ | ND | ND |
| Beskow et al. (2001) | Sweden | PCR | 440 CIS; 476 controls | 0 | ++ | -- |
| Lin et al. (2001) | Senegal | PCR | 55 Cancers; 190 controls | 0 | – | – |
| Wang et al. (2001) | Costa Rica | PCR | 166 HSIL/Cancers; 320 LSIL/HPV; 173 HPV-controls | 0 | 0 | -- |

++, OR significantly > 1.0; --, OR significantly < 1.0; +, OR not significant but $OR \geq 1.5$; -, OR not significant but $OR \leq 0.7$; 0, OR not significant and $0.70 < OR < 1.5$. Findings in either overall or HPV-16 restricted analyses were evaluated.

^a IWC, Controls from the International Histocompatibility Workshop.

^b For serological testing, DQB1*0301-3 was represented by serogroup DR2; DRB1*1501 was represented by serogroup DR2; and DRB1*13 was represented by serogroup DR6.

^c LR, low resolution genotyping.

^d ND, Not done.

^e Inferred based on authors' affiliations.

in US Hispanics with a statistically significant odds ratio of 2.9 for invasive cancer (4.8 in analysis restricted to HPV16 positive subjects), measuring HLA by PCR (Apple et al., 1994). Since then, some studies have reported a more modest 2-fold increase in cancer for Brazilian women (estimate adjusted for age and ethnicity) and for European Caucasian women (Sanjeevi et al., 1996; Helland et al., 1998; Krul et al., 2000; Beskow et al., 2001; Maciag et al., 2000). Again, in studies where effects were observed, they were often strengthened in analysis restricted to HPV-16, confirming the type specificity of associations involving acquired immune responses (Apple et al., 1994; Maciag et al., 2000; Apple et al., 1995). However, many studies did not report an association between DRB1*1501 or DQB1*0602 with cervical cancer (Wang et al., 2001; Odunsi et al., 1996; Sanjeevi et al., 1996; Wank and Thomssen, 1991; Helland et al., 1994; Gregoire et al., 1994; Glew and Stern, 1992; Brady et al., 1999; Lin et al., 2001; Ferrera et al., 1999; Montoya et al., 1998), and a study conducted within a large cohort of US Caucasian women has conversely reported a significant protective effect for these alleles (Hildesheim et al., 1998).

Thus, the evidence suggests that DRB1*13 and/or DQB1*0603 are likely to protect against the development of cervical cancer while no alleles have consistently been shown to increase risk of disease. In addition to the three groups of alleles discussed above, numerous other HLA Class II alleles were reported to be associated with cervical neoplasia in at least one publication, but since no consistency between studies was observed for these other alleles they are not discussed further in this review. It should be noted, however, that because many of the studies conducted to date were modest in size, alleles with low prevalence in any given population were difficult to assess, and their varying prevalence across populations may make consistent findings difficult to observe. The identification of the few consistent findings as illustrated above are therefore of great significance in light of the multiple challenges inherent in identifying such HLA allele/haplotype-disease associations.

It is of interest that the only Class II alleles found consistently to be associated with cervical

neoplasia were protective alleles. This suggests the possibility that protective alleles are more easily discernable in epidemiological studies than alleles that confer risk. Based on the available data, one might hypothesize that while a single HLA allele capable of recognizing, binding, and efficiently presenting an HPV antigen to the immune system might suffice to reduce cancer risk, a combination of several alleles unable to efficiently present HPV antigens to the immune system are required before increases in risk are demonstrable at the individual level.

2.3. *Studies of HLA Class I genes and risk of cervical neoplasia*

Many fewer studies have evaluated the role of HLA Class I alleles in cervical cancer pathogenesis. The few studies that have systematically evaluated HLA Class I alleles and risk of cervical neoplasia have been conducted in Caucasian populations in Europe and have largely relied on serological typing methods that do not permit high resolution, specific assessment of individual genotypes (Glew et al., 1993; Krul et al., 2000; Brady et al., 1999). In one of these studies (Brady et al., 1999), a protective effect of B15 was observed, but this was not confirmed in the other studies (Glew et al., 1993; Krul et al., 2000). Similarly, one study (Bontkes et al., 1998a,b) reported a positive association between B44 and risk of disease progression that was not confirmed by others (Glew et al., 1993; Krul et al., 2000; Brady et al., 1999). Two studies conducted in two distinct populations in North and Central America suggested that B7, in combination with DQB1*0302, was associated with elevated risk of cervical disease (Wang et al., 2001; Hildesheim et al., 1998). It will be interesting to see whether these findings are confirmed in future studies.

Studies that systematically evaluate the association between HLA Class I genes and cervical cancer using allele-specific, high-resolution genotyping methods are just now beginning to be reported. The first such study conducted in three populations in Central and North America reported a consistent negative association between HLA C*0202 and disease risk in all three popula-

tions studied (Wang et al., 2002). This effect was not HPV-type-specific, suggesting the involvement of the innate (which is not driven by specific foreign antigens) rather than acquired immunity (which is driven by specific foreign antigens presented by HLA) as an explanatory factor. This finding requires confirmation in future studies.

2.4. *Unresolved issues and future directions*

Issues that have not adequately been resolved to date and that should be addressed in future work include but are not limited to the following: (1) evaluation of HPV type-specificity (and variant-specificity; see below) of observed effects; (2) evaluation of the independent effects of HLA alleles in linkage disequilibrium, and (3) evaluation of the possibility of true differences in effects (qualitative interaction) in different studies due to differences in linkage disequilibrium patterns in different ethnic groups. Additional studies of HLA Class I alleles are acutely needed to confirm or deny claims made by the few studies published to date.

Given the diversity of the HLA genes and the fact that most HLA alleles occur with relative infrequency and in strong linkage disequilibrium with other alleles, future studies of HLA and cervical cancer will need to be large and/or involve pooling of existent studies if we are to further our understanding of the link between HLA and cervical neoplasia. Moderately sized studies such as those reported to date have low power to detect true associations and are prone to false positive findings resulting from the multiple comparisons made (Garcia-Closas and Lubin, 1999; Garcia-Closas et al., 1999; Browner and Newman, 1987). Future pooling efforts will need to carefully consider issues of population stratification (i.e. confounding by ethnicity), in light of the fact that the distribution of HLA alleles is known to correlate with ethnicity, and ethnicity is a known determinant of disease risk in many populations.

As an alternative to larger studies, efforts to identify biologically relevant HLA groupings might also be undertaken. Such efforts would increase study power by virtue of increasing the

frequency with which the relevant groups are observed in the populations studied. As an example, it has been proposed that individual HLA alleles could be grouped based on the conformational ‘motif’ of their antigen binding site (Odunsi and Ganesan, 2001). Using this approach, all HLA alleles that have a similar antigen binding groove motif would be combined into a single group and evaluated jointly. Finally, given the hypothesis that innate immunity and natural killer cell responses might be important in cervical cancer pathogenesis, HLA Class I alleles could be combined into groups defined based on their ability to bind different forms of the Class I receptors found on natural killer cells (known as KIR receptors).

3. HPV variants

In this section, we review epidemiological studies that have evaluated the association between HPV and risk of cervical neoplasia. We will not review laboratory studies that have evaluated possible functional differences between different variants of HPV. Evaluation of the functional significance of variants found to correlate with disease is still in its infancy, and the evidence that is currently available has focused largely, although not exclusively, on HPV16 (Choo et al., 2000; Hsieh et al., 2000; Grassmann et al., 1996; Kammer et al., 2000; Schmidt et al., 2001; Stoppler et al., 1996; Tornesello et al., 2000; Veress et al., 2000).

3.1. Background

It is known that of the more than 40 HPV types that infect the genital tract, only a subset are involved in cervical cancer pathogenesis. This includes most notably HPV16, which accounts for approximately 50% of all cervical cancer cases worldwide. Different types of HPV are defined as having > 10% variation in specified regions of the viral genome (Heinzel et al., 1995; Stewart et al., 1996; Xi et al., 1997). Viruses varying from each other by 2–10% are referred to as subtypes and are infrequently observed, while those differing by < 2% are more frequently seen and referred to as

intratypic variants. Given that different types of HPV have been shown to have different oncogenic potential, it is reasonable to hypothesize that variants of specific oncogenic types might also have differential oncogenicity.

Early work that evaluated intratypic variation within HPV16 and other HPV types noted that the distribution of HPV variants varied considerably in different geographical regions, suggesting that the virus and the host have co-evolved over time (Heinzel et al., 1995; Stewart et al., 1996; Ho et al., 1993; Ong et al., 1993; Yamada et al., 1995, 1997). For HPV-16, five phylogenetic lineages of the virus have been defined, and are classified based on their suspected origin as European (E), Asian (As), Asian–American (AA), African-1 (Af1), and African-2 (Af2). This phylogenetic classification was determined based on nucleotide sequence analysis of the long control region (LCR) of the virus.

3.2. HPV16 variants and their association with cervical neoplasia

Numerous epidemiological studies have been conducted to date to evaluate the association between HPV intratypic variants and cervical neoplasia (Brady et al., 1999; Schmidt et al., 2001; Xi et al., 1997; Terry et al., 1997; Bontkes et al., 1998a,b; Zehbe et al., 1998; Nindl et al., 1999; Etherington et al., 1999; Luxton et al., 2000; Andersson et al., 2000; Bible et al., 2000; Zehbe et al., 2001; Hu et al., 2001; Pang et al., 2002; Villa et al., 2000; Matsumoto et al., 2000; Berumen et al., 2001; Hildesheim et al., 2001, 2002). While initial work conducted largely in homogeneous European populations in Sweden, Russia, Italy, UK, Germany, The Netherlands, and Poland provided conflicting data on the link between intratypic variation in HPV16 and disease risk, it is now commonly accepted that this was due to the overwhelming predominance of the lower risk European lineage of the virus in the populations studied. In these European studies, variability within the European lineage was not consistently found to be associated with disease risk (Brady et al., 1999; Schmidt et al., 2001; Terry et al., 1997; Bontkes et al., 1998a,b; Zehbe et al., 1998; Nindl et al., 1999; Etherington et al., 1999; Luxton et al.,

2000; Andersson et al., 2000; Bible et al., 2000; Zehbe et al., 2001; Hu et al., 2001; Pang et al., 2002). None of these studies, however, had the ability to evaluate risk associated with non-European HPV16 variant infections. Subsequent studies conducted in more diverse populations primarily in North and Latin America have demonstrated a more clear and reproducible pattern of risk associated with infection by non-European HPV16 variants (Xi et al., 1997; Villa et al., 2000; Matsumoto et al., 2000; Berumen et al., 2001; Hildesheim et al., 2001, 2002) (Table 2). When individuals infected with non-European variants of HPV16 were compared with those infected with the European variant of the virus, infection with the non-European variants was found to be associated with a 2–9-fold increased risk of cervical cancer and high-grade cancer precursors. Findings were consistent in the case-control and longitudinal studies. Furthermore, similar results were observed in a study conducted in Asia, where both the higher risk Asian and lower risk European variants are present in the population (Matsumoto et al., 2000). It should be noted, that while it appears that individuals infected with non-European variants of HPV16 are at higher risk of disease than those infected with the European variant, compared with HPV negative subjects and those infected with non-oncogenic HPV types, women infected with the European variant of HPV16 are still at a greatly increased risk of disease (Hildesheim et al., 2001).

3.3. Association with disease of HPV variants other than HPV16

While most studies that have examined intratypic variants have focused on HPV16, a few studies to date have begun to evaluate another HPV type, namely HPV18 (Villa et al., 2000; Hecht et al., 1995; Lizano et al., 1997). Studies of HPV18 variants are anecdotal at this point, given the relative rarity of this type and the small size of studies conducted to date. However, consistent with the HPV16 studies, studies of HPV18 suggest a possible link between non-European variants and disease risk. In one study conducted in the United States, two out of three HPV18 positive

cervical cancers contained the Asian/Amerindian (As/Ai) variant of HPV18 (the prototypic HPV18 virus) compared with none of 12 HPV18 precursor lesions (Hecht et al., 1995). Interestingly, reports that have examined the association between HPV variants and cervical adenocarcinomas, tumors that have been shown to be preferentially linked to HPV18 infections, have demonstrated a predilection of Asian–American (for HPV16) and Asian–Amerindian (for HPV18) variants for this tumor type (Berumen et al., 2001; Lizano et al., 1997). In one study conducted in Mexico (Lizano et al., 1997), 75% of cervical adenocarcinomas or adenosquamous carcinomas positive for HPV16 or HPV18 were infected with a non-European variant of HPV, compared with less than half (47%) of 19 HPV16 or HPV18 positive squamous cell tumors. If confirmed, this may explain the unique distribution of HPV types seen in these rare, glandular tumors.

3.4. Unresolved issues and future directions

While results to date appear to clearly indicate a role for HPV variants in the development of cervical cancer, at least one important concern remains. Since it is known that HPV variants correlate with geographical origin, and therefore, with ethnicity, confounding by ethnicity (also referred to as population stratification) could potentially account for the excess risk observed for non-European variants in some studies. Some of the studies conducted to date have attempted to control for this through statistical adjustment (Xi et al., 1997; Villa et al., 2000). In these studies, residual confounding cannot be ruled out as an explanatory factor. However, in at least one study, genotyping of individuals with the European and non-European HPV16 variants confirmed the lack of ethnic differences between the two groups, suggesting that the observed association between non-European HPV16 and disease is real (Hildesheim et al., 2001). Nonetheless, future studies of HPV variants and cervical neoplasia should attempt to carefully control for this potential confounding factor.

Future studies are also needed to more clearly examine the role of intratypic variants of HPV

Table 2
Summary of studies that have evaluated the association between non-European HPV16 intratype variants and cervical neoplasia

| Authors | Country | Typing method | Region typed | Study groups (all HPV16 positive) | RR ^a | Design |
|--------------------------|------------|---------------|--------------|--|---|-------------------------------|
| Xi et al. (1997) | USA | SSCP | LCR | 57 University health clinic attendees (nine developed CIN/3); 66 STD clinic attendees (ten developed CIN2/3) | 6.5* (Univ Hlth Clinic); 4.5 (STD clinic) | Longitudinal |
| Villa et al. (2000) | Brazil | Sequencing | LCR | 51 Screening clinic attendees (three progressed to HSIL) | 3.8 ^b | Longitudinal |
| Matsumoto et al. (2000) | Japan | Sequencing | E6 | 43 Cancer cases; 40 CIN1-3 controls | 4.1* ^c | Case-control |
| Berumen et al. (2001) | Mexico | Sequencing | E6-L1 | 92 Cancer cases; 20 normal controls | 7.9* | Case-control |
| Hildesheim et al. (2001) | Costa Rica | Sequencing | LCR | 16 Cancer cases; 56 HSIL cases; 87 LSIL/normal controls | 8.9* (Cancer); 2.2 (HSIL) | Population-based case-control |

*, *P*-value < 0.05.

^a RR for cervical neoplasia comparing non-European to European HPV16 infection.

^b Estimated from numbers presented in the manuscript.

^c Authors classified all individuals with HPV infections that varied at 1 + nucleotide position from the prototypic European variant into the variant group, thus including some European-like variants in their variant grouping. Infection with the Asian lineage was the predominant type seen in the variant grouping used by the author.

types other than type 16, as well as the role of HPV variants in histological subtypes of cervical cancer, particularly cervical adenocarcinomas. To provide adequate power to evaluate these issues, studies will need to be large and carefully designed, as already discussed in the HLA section of this review. Finally, since HPV variants may have differential risk of disease due to differences in the immune handling of different variant forms of the virus by the host, evaluation of the joint effect of HLA and HPV variants should be considered, but only in studies that are sufficiently large to provide adequate power to detect joint effects. Some studies have attempted to do this, but have been too small to provide any conclusive results (Hildesheim et al., 2001; Zehbe et al., 2001).

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