Infection, Genetics and Evolution 21 (2014) 220–226

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Review

Genomic diversity of human papillomaviruses (HPV) and clinical implications: An overview in adulthood and childhood

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ARTICLE INFO

Article history:
Received 3 September 2013
Received in revised form 1 November 2013
Accepted 6 November 2013
Available online 26 November 2013

Keywords:
Human papilloma viruses
HPVs
Genomic diversity
Adults
Children

ABSTRACT

During the last years, several researchers have highlighted the importance of characterizing more than one genomic region in order to detect recombination and classify variants of human papillomaviruses (HPVs) properly. HPVs variants differ in their biological, molecular and chemical properties. Therefore, this genomic diversity can present differences in the natural history and pathogenicity of HPVs. Different 'high-risk' HPVs variants of the genotypes HPV 16 and 18 can confer varied risks of viral persistence in the human cervix and influence HPVs progression to cervical cancer. Moreover, different 'low-risk' HPVs variants of the genotypes HPV 6 and 11 can play a unique role in the development of anogenital and cutaneous warts, recurrent respiratory papillomatosis (RRP) and ophthalmic pterygium. In future, the precise impact of genomic HPVs diversity to the clinical course of HPVs-associated diseases as well as to the efficacy of the current HPVs vaccines remains to be elucidated.

Contents

1. Introduction

Human papillomaviruses (HPVs) are small non-enveloped double-stranded DNA viruses that belong to the *Papillomaviridae* family. At the beginning of the 1980s, the first HPVs were isolated and cloned using molecular techniques and this initiated a rapid expansion of molecular and epidemiological studies, which, just one decade later, impressively supported HPVs as the principal causative factor for cervical cancer (zur Hausen, 2002). This link had initially been suspected by Professor Harold zur Hausen, who was convinced by the early 1970s that HPVs infections are associated with cervical cancer. Almost 40 years later, Professor Harold zur Hausen received the Nobel Prize in Physiology and Medicine for 2008 and his observation provided the background for attempts to develop human vaccines against 'high-risk' HPVs, including genotypes HPV 16 and 18, using virus-like particles (VLPs). Since 2007, specific vaccination programs have been

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http://dx.doi.org/10.1016/j.meegid.2013.11.002

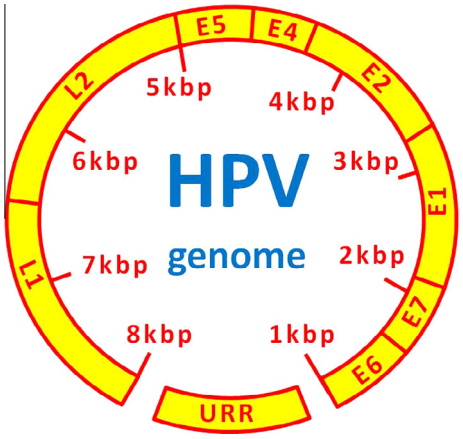

Fig. 1. HPV DNA genome: a schematic presentation.

implemented into clinical practice, while research on the next generation of multivalent VLPs-based vaccines against a wider array of HPVs continues.

HPVs are epitheliotropic and infect the cutaneous or mucosal epithelial cells, exclusively. After entering the host cells of the epithelial basal layer, replication of the virus occurs in the nuclei of the infected cells and the production of mature virions occurs in the suprabasal epithelial cell layers (Bonnez, 2002). HPVs infection is highly transmissible with a variable incubation period and can cause a diverse range of epithelial lesions. It can culminate in latent infection, which is insufficient to support transmissibility; in subclinical infection, which is active, but without clinical signs; or in clinical infection leading to benign epithelial lesions or malignant neoplasms. The majority of latent, subclinical and clinical manifestations of HPVs infection are capable of undergoing spontaneous resolution.

The oncogenic role of HPVs has been well-established by the regular presence of HPVs DNA in cervical cancer biopsy specimens as well as by the precise identification of the transforming properties of the E6 and E7 viral proteins that interact with the growth-regulating host-cellular proteins (Burd, 2003). In addition to cervical cancer, a major proportion of anal, perianal, vulvar and penile cancers appear to be primarily linked to HPVs. Moreover, extended research during the last decade has also led to the identification of HPVs in other non-genital cancers, such as breast and lung cancer (Mammas et al., 2011).

All HPVs have the same general organization of their genome, as shown in Fig. 1. Their genome consists of a single molecule of double-stranded, circular DNA containing approximately 7900 bp, which is functionally divided into three regions (Baker et al., 1991; Sapp et al., 1995). The first is a non-coding upstream regulatory region (URR) of 400–1000 bp. This region contains the p97 core promoter along with enhancer and silencer sequences that regulate DNA replication by controlling the transcription of the open reading frames (ORFs) (Apt et al., 1996). The second is the early (E) region that carries early ORFs encoding for the non-structural regulatory proteins E1, E2, E4, E5, E6 and E7, which are involved in viral replication, transcription, transformation, adaptation and oncogenesis. The third is a late (L) region, encoding the major capsid protein L1 and the minor capsid protein L2, which are structural proteins forming the icosahedral viral capsid comprising 72 capsomers (Baker et al., 1991; Sapp et al., 1995). The URR encompassing the origin of replication, the E6/E7 gene promoter, enhancers and silencers, is located between the early (E) and the late (L) regions (Longworth and Laimins, 2004).

2. Genomic diversity of HPVs

Different genotypes of HPVs are defined by a genomic sequence dissimilarity of more than 10% in their nucleotide sequences in the E6, E7 and L1 ORFs (Van Ranst et al., 1993; de Villiers, 2013; Bernard et al., 2013). Isolates within the same genotype differing by 0–2% in their sequences compared with the reference sequence are referred to as HPVs variants and those differing by 2–10% are referred to as subtypes. The URR region of HPVs contains the highest degree of genomic diversity (Apt et al., 1996). HPV 16 and 18 genomic sequences create evolutionary trees with the bifurcation driven by variants with a high prevalence in cohorts from different regions of the world (Ho et al., 1993; Ong et al., 1993). This evolutionary diversity is reflected in the phylogeny of these strains and is reminiscent of the migration patterns of *Homo sapiens*. Thus, it has been suggested that HPVs variant lineages may have co-diversified with human populations as they exponentially expanded across the planet.

During the last two decades, research on the genomic diversity of the genotypes HPV 16 and 18 was initially inferred from the URR and E6 sequences and has been recently expanded to include the complete genomes (Chen et al., 2005,2009; Arias-Pulido et al., 2005a,b). Genomic diversity of the HPV 16 genotype has been largely studied and to date different genomic variants have been described (Icenogle et al., 1991; Yamada et al., 1997; Kurvinen et al., 2000), including the Asian (As), Asian-American (AA), African 1 (Af-1), African 2 (Af-2) and the European (E), as well as a recent variant of North American 1 (NA1). These variants have been identified based on nucleotide changes in the E6, L1 ORFs and the URR and have been found in different biological environments and geographical locations (Icenogle et al., 1991). Among the ethnic groups of Africans, Asians and Caucasians, the European variants are usually found in all regions except Africa. The Asian variants are a subclass of the European lineage and are commonly detected in South-East Asians (Yamada et al., 1997).

HPV 18 genomic variants are grouped into three main branches: the Asian-American (AA), the European (E) and the African (Af) (Arias-Pulido et al., 2005a,b). These three branches have been equally distributed among controls and cases and stratified by Hispanic and non-Hispanic ethnicities. Among invasive cervical cancer cases, no significant differences in the three HPV variant branches have been observed among ethnic groups or when stratified by histopathology. The African (Af) branch has shown the greatest nucleotide variability when compared to the HPV 18 reference sequence and has been more closely related to the genotype HPV 45 than either the Asian-American (AA) or European (E) branches. These variants have been identified based on nucleotide changes in the E6, L1 ORFs and the URR.

Detailed sequence analysis of genotypes HPV 6 and 11 of the E7, E1, E2, E4, L2 ORFs and the URR has revealed the existence of several genomic variants (Burk et al., 2011; Kocjan et al., 2011). Phylogenetic analysis of complete genomes derived from published HPV 6 variants has suggested the presence of two deeply separate branches for HPV 6, with the reference genome HPV 6b forming lineage A and reference genomes HPV 6a and 6vc forming lineage B (Burk et al., 2011). Within the HPV 6 lineage B, there are three sublineages with HPV 6vc belonging to sublineage B1 and HPV 6a belonging to sublineage B3. It has been found that HPV 11 variants are more highly conserved, thereby not meeting the criteria for classification into more than one lineage (Burk et al., 2011). The nomenclature proposed for the HPV 11 lineage is based on two branches: sublineage A1, which includes variants clustering with the HPV 11 reference genome and sublineage A2, which includes all other variants.

Subsequent studies have also investigated the genomic diversity of other HPV genotypes, including HPV 2, HPV 5, HPV 8, HPV 27, HPV 31, HPV 33, HPV 35, HPV 44, HPV 52, HPV 53, HPV 56, HPV 57 and HPV 66 (Heinzel et al., 1995; Maver et al., 2011; Deau et al., 1993, 1991; Chan et al., 1997; Calleja-Macias et al., 2004, 2005a,b; Prado et al., 2005; Gagnon et al., 2007, 2004, 2005; Raiol et al., 2009; Stewart et al., 1996).

The importance of the genomic diversity of HPVs is related to several issues, including epidemiology, clinical management and prevention strategies (Bernard et al., 2006). Despite phylogenetic similarities, HPVs variants can differ in their natural history and pathogenicity. Indeed, different HPVs variants are functionally diverse in their biological, molecular and chemical properties. Comparisons of different genomic HPVs variants have confirmed that each HPVs variant can have a specific manifestation and clinical course. Moreover, it is crucial to investigate the impact of genomic HPVs diversity on the efficacy of the current as well as future vaccination programs against HPVs. Nevertheless, it should be highlighted that this impact is not fully predictable at the introduction of any vaccine, because it varies according to many variables that are not strictly related to immune response only.

3. Evolution of HPVs genomic diversity

Different HPVs genotypes and variants were already in existence when the human species was formed (Bernard et al., 2006). HPVs have maintained their basic genomic organization for more than 100 million years and have remained stable over time with unexpected major variations (Xi et al., 2006; Bernard et al., 2006). Unlike other viruses, HPVs genotypes have evolved very slowly and have diverged since the origin of humanity at a slow evolutionary rate estimated to be 10^{-8} base substitutions per site per year (Chen et al., 2009). The HPVs variants of each genotype form phylogenetic trees and variants from specific branches are often unique to specific ethnic groups (Bernard et al., 2006). Immigrant populations contain mixtures of variants depending on their respective ethnic origins.

Evolution of HPVs genotype variation over time is a crucial factor in estimating the clinical course of HPVs infection. This can predict the prognosis of both benign and malignant lesions associated with HPVs infection and can indicate the recommended management strategies. Moreover, it can influence the long-term effectiveness of current vaccines against HPVs. Based on their stability the effectiveness of current vaccines is fundamentally predictable. However, the large number of different variants of HPVs genotypes raises the question as to the number of HPVs that must be included in the preparation process of the next generation of multivalent vaccines against HPVs.

4. Clinical manifestations

4.1. Cervical cancer

Cervical cancer is the second most common cancer among women worldwide, with almost 260,000 deaths, annually (Castellsagué, 2008). The disease disproportionately affects the poorest regions of the planet as more than 80% of cases are found in Latin America, sub-Saharan Africa and the Indian subcontinent. Persistent infection by certain oncogenic HPVs genotypes is firmly established as a necessary cause of cervical cancer (Schiffman et al., 2007). There are four major steps involved in cervical cancer development including infection of metaplastic epithelium at the cervical transformation zone, viral persistence, progression of persistently infected epithelium to cervical pre-cancer, and invasion through the basement membrane of the epithelium. Infection is extremely common in young women in their first decade of sexual activity. Each HPVs genotype acts as an independent infection, with differing carcinogenic risks linked to evolutionary species. Based on the epidemiologic classification in terms of their risk to induce cervical cancer, HPVs can be divided into 'low-risk' genotypes, including HPV 6, HPV 11, HPV 40, HPV 43 and HPV 44 and 'high-risk' genotypes, including HPV 16, HPV 18, HPV 31, HPV 33, HPV 35, HPV 39, HPV 45, HPV 51, HPV 52, HPV 56, HPV 58, HPV 59, HPV 68, HPV 73 and HPV 82 (Burd, 2003). HPV 16 and 18 are the two most common 'high-risk' genotypes causing approximately 70% of all cervical cancers, worldwide.

One of the potential risk factors for persistence and progression of HPVs infection can involve the genomic diversity of HPVs. Different HPVs variants are diverse in their biological, molecular and chemical properties and therefore, this may cause significant differences in their natural history and pathogenicity. In fact, persistent HPVs infection and intraepithelial neoplasia are established typically within 5–10 years, from less than 10% of new infections. Invasive cancer arises over many years, even decades, in only a minority of women with intraepithelial neoplasia, with a peak or plateau in risk at about 35–55 years of age (Burd, 2003; Castellsagué, 2008). Studies on multiethnic populations have also shown that there is a three-fold or greater risk of cervical cancer for the Asian-American (AA) or the African (Af) HPV 16 variants compared to the European (E) variants (Xi et al., 2006; Sichero et al., 2007; Hildesheim et al., 2001). Asian-American (AA) genomic variants of HPV 16 strongly stimulate p53 degradation and increase p97 promoter activity compared with the HPV 16 prototype (Stöppler et al., 1996; Veress et al., 2001). Several researchers have also shown that non-European HPV 16 variants are associated with an increased risk of HPVs persistence and immunogenicity and pose a two- to nine-fold increased risk of high-grade intraepithelial neoplasia and cervical cancer depending on the respective populations (Villa et al., 2000; Hildesheim and Wang, 2002). These variants can express differences in transcriptional regulation, biological activity and immune response to specific viral epitopes (Veress et al., 2001).

Different HPV 16 E6 variants interact differently with cellular proteins involved in host-cellular transformation, control of cell cycles and apoptosis. The E6 variants regulate oncogenesis by the Notch signaling pathway and oncogenic Ras differentiation, suggesting that they could feasibly enhance the oncogenic potential of the Asian-American (AA) variants (Chakrabarti et al., 2004). Persistence of HPV 16 may be associated with variants exhibiting a non-synonymous substitution at nucleotide position T350G (Londesborough et al., 1996). Destruction of the E2 gene induces E6/E7 oncogene transcription during viral integration in the viral genome prototype whereas, in the Asian-American (AA) variants, the E2 gene is intact which may account for the higher replication efficiency in comparison with the European (E) variants (Jeon et al., 1995). The Asian-American (AA) variants exhibit a three-fold increase in p97 promoter activity compared to the European (E) variants (Casas et al., 1999).

HPV 18 has been associated with both recurrent lesions with very bad clinical prognosis and benign lesions (Xi et al., 2006; Sichero et al., 2007; Hildesheim et al., 2001). This fact can reflect oncogenic potential differences among HPV 18 variants due to their genomic diversity. Hecht et al. identified an HPV 18 variant with lower oncogenic potential due to its absence in cervical cancer, but presence in almost 40% of intraepithelial lesions (Hecht et al., 1995). Villa et al. have found that the non-European HPV 18 variants persisted more frequently and were more associated with pre-invasive lesions (Villa et al., 2000). The genomic diversity of HPV 16 and 18 can also explain their geographic variations and prevalence of the pre-dominant genomic variants (Sichero et al., 2007).

4.2. Anogenital warts

In adulthood, anogenital warts (condylomata acuminate) are the most common sexually transmitted infection (Garland et al., 2009). In children, the implications of anogenital warts are highly controversial as regards sexual abuse (Mammas et al., 2009). Treatments, including chemical, physical and immunological management are lengthy, expensive, inconvenient and often painful. Recurrence is frequent due to HPVs persistence in perilesional skin.The 'low-risk' genotypes HPV 6 and 11 are responsible for more than 90% of benign anogenital warts cases (Garland et al., 2009; Komlos et al., 2012; Hawkins et al., 2013).

Genomic variation of HPV 6 and 11 isolates from anogenital warts specimens has been determined by sequencing the E6 and E7 ORFs and the URR (Krige et al., 1997; Danielewski et al., 2013). The majority of HPV 6 variants in anogenital warts cluster to the HPV 6 sublineage B1 (Danielewski et al., 2013). The occurrence of multiple concurrent anogenital warts is a consequence of infection with a single HPV 6 genomic variant, rather than infection with multiple genomic variants of HPVs (Komlos et al., 2013).

4.3. Cutaneous warts

Cutaneous warts, including common (verruca vulgaris), flat (verruca plana), intermediate, mosaic, periungual and plantar (verruca plantaris) warts, are benign epidermal proliferations varying in appearance, size and shape (Jablonska et al., 1997). They represent a common skin condition in children, with a peak incidence in the teenage years and a sharp decline thereafter. Their clinical course can range in severity from a minor nuisance that resolves spontaneously to a chronic condition constituting a significant burden for immunocompromised adults, such as organ transplant recipients (Gassenmaier et al., 1986). Numerous treatments for cutaneous warts are currently available, although no single therapy has been established as completely curative (Mulhem and Pinelis, 2011). Cutaneous warts have been associated with HPV 1, HPV 2, HPV 3, HPV 6, HPV 7, HPV 10, HPV 11, HPV 27, HPV 28, HPV 29, HPV 40, HPV 41, HPV 43, HPV 57, HPV 63, HPV 77, HPV 91, HPV 94, and HPV 117, with HPV 1 being the most prevalent, followed by HPV 27, HPV 57 and HPV 2 (Schmitt et al., 2011). Different cutaneous warts are related to different HPVs. HPV 1, HPV 2, HPV 3, HPV 4, HPV5, HPV 7, HPV 27 and HPV29 are detected in common warts; HPV 3, HPV 10, HPV 28 and HPV 29 in flat warts; HPV 2, HPV 3, HPV 10, HPV 28 and HPV 29 in intermediate warts; HPV 2 in mosaic warts; HPV 1, HPV2, HPV 4, HPV 5, HPV 7, HPV 27 and HPV 57 in periungual warts; and HPV 1, HPV 2, HPV 3, HPV4, HPV 27, HPV 29 and HPV 57 in plantar warts (Schmitt et al., 2011).

Identification of different HPVs variants in cutaneous warts can be useful for the prediction of the clinical course of the lesion or which lesion is or is not resolved spontaneously (Bruggink et al., 2012). For instance, cutaneous warts containing HPV 1 variants have revealed the most distinct clinical profile, being related to children aged less than 12 years, plantar location, duration less than 6 months and to patients with less than 4 warts (Bruggink et al., 2012). The development of cutaneous warts is accompanied by angiogenesis and this process can be independently regulated by the presence of specific HPVs variants (Harada et al., 2000). Moreover, HPVs genomic diversity analyses can be useful for future vaccine strategies against cutaneous HPVs and to estimate the efficacy of these vaccines in immunocompromised patients. However, to date, identifying the HPVs variant does not influence the treatment of choice or the management strategy of cutaneous warts (Sterling et al., 2001; Mulhem and Pinelis, 2011; Bruggink et al., 2012).

4.4. Recurrent respiratory papillomatosis

Recurrent respiratory papillomatosis (RRP) is a potentially life-threatening disease that occurs in both children and adults (Goon et al., 2008; Donne et al., 2010). HPVs exposure to newborns has been suggested to predominantly occur via maternal genital infection, although the frequency and routes for both vertical and horizontal transmission remain controversial (Mammas et al., 2009). RRP generally presents with progressive hoarseness and stridor connected to the growth of multiple exophytic lesions within the larynx, although lesions may also occur at other sites within the oropharyngeal and lower respiratory tract. The clinical course of RRP can vary from spontaneous remission, to relatively stable lesions, to aggressive cases that can potentially be life-threatening causing severe respiratory obstruction. RRP can have a devastating impact on affected children due to the number and frequency of surgical procedures required to control their disease, with some patients requiring hundreds of operations in their lifetime. Malignant transformation to squamous cell carcinoma in RRP is a rare outcome and is often a consequence of irradiation, bleomycin chemotherapy or cigarette smoking, albeit it can also develop without any known carcinogenic risk factors (Goon et al., 2008). Although there is a general consensus that the HPV 11 genotype results in more aggressive disease compared to HPV 6, the clinical differences between HPV 6 and 11 disease may not be accurately predictable as a number of variants of these viruses exist (Donne et al., 2010). RRP tissue may contain more than one HPVs variant or even be co-infected with other viruses that may affect the clinical outcome (Donne et al., 2010).

To date, several studies have investigated the presence of different variants of HPV 6 and 11 genotypes in RRP patients (Grassmann et al., 1996; Kovelman et al., 1999; Gáll et al., 2011, 2013; Kocjan et al., 2011, 2013; Combrinck et al., 2012, 2013; Chansaenroj et al., 2012; de Matos et al., 2013). Notably, the frequent recurrence of RRP is a consequence of long-term persistence of the identical initial HPV genomic variant (Kocjan et al., 2013). Initially, it was supported that it was not possible to correlate different HPVs variants with disease severity (Kovelman et al., 1999; de Matos et al., 2013). In the study by Kovelman et al, it was found that amino acid differences observed between HPV 6 variants of the E2 protein do not affect its viral function (Kovelman et al., 1999). However, the genomic diversity of HPV 6 identified by Combrinck et al. based on genome changes within the 712–991 bp region encompassing the URR, with variations in length resulting from insertions and duplications and the 453-bp gene encoding the E6 protein, was correlated with disease severity (Combrinck et al., 2012). Recently, Gáll et al. described different alterations resulting in amino acid changes in the E1, E2 and L1 ORFs of HPV 11 that are exclusively present in moderately aggressive disease or severe RRP (Gáll et al., 2013). Moreover, the molecular analysis of different HPV 6 variants revealed a high sequence variability within the E6 ORF, enabling the detection of amino acid changes unique to isolates from carcinomas (Grassmann et al., 1996). Further studies should be conducted to clarify the exact role of different HPVs variants in order to identify the tendency of RRP to regress spontaneously or to predict its malignant transformation dynamic.

4.5. Ophthalmic pterygium

Pterygium is a wing-shaped fibrovascular lesion of the ocular surface that may occur in both adults and children (Eze et al., 2011). It can cause irregular astigmatism, cornal scarring, restriction of ocular motility or chronic ocular surface inflammation. Treatment is exclusively surgical, however, pterygium often tends to recur aggressively. Although pterygium is considered as a

sun-related disease, several studies have suggested that several co-factors, including repeated microtrauma, mediated by exposure to dust, chronic conjunctival inflammation, genetic predisposition, ocular dryness as well as HPVs infection may also be involved in the pathogenesis of pterygium (Detorakis and Spandidos, 2009).

The presence of HPVs genotypes in pterygium has been reported by several studies, with rates ranging from very low to almost 100% of cases (Gallagher et al., 2001; Chalkia et al., 2013; Di Girolamo, 2012). This variation can be explained only partially by study design or molecular techniques errors (Piecyk-Sidor et al., 2009). The major differences in the frequency of HPVs in geographically distant populations can be explained by the presence of specific HPVs variants (Piras et al., 2003). Overall, although current data suggest that HPVs variants are not necessary to initiate the pathogenesis of pterygium, HPVs infection may be a significant co-factor in susceptible hosts (Tsai et al., 2009). It has been well demonstrated that HPV 16 and 18 E6 variants contribute to HPVs-mediated pterygium pathogenesis, which is partly involved in p53 inactivation in HPVs DNA-positive pterygium (Tsai et al., 2009). Persistent conjunctival HPVs variants may play a role in the recurrence of pterygia post-excision. However, larger studies are required to elucidate this hypothesis (Gallagher et al., 2001).

5. Vaccines against HPVs

The current HPVs vaccines target the most prevalent and aggressive oncogenic 'high-risk' HPVs genotypes associated with cervical cancer, HPV 16 and HPV 18 (Ahmed et al., 2013). Quadrivalent vaccines also target against the most prevalent 'low-risk' HPVs associated with anogenital warts and RRP, HPV 6 and HPV 11. Both vaccines comprise virus-like particles (VLPs), based on the major capsid protein L1 and vaccine-induced type-specific protection is likely mediated by neutralizing antibodies targeting L1 surface-exposed domains. VLPs have demonstrated almost 100% efficacy against high-grade intraepithelial lesions and cervical cancer associated with HPV 16 and 18 genotypes in clinical trials (Lu et al., 2011). The abovementioned HPVs vaccines also afford a significant degree of cross-protection against other HPVs not included in the vaccines (Lu et al., 2011). Malagón et al. conducted a systematic comparison of the cross-protective efficacy induced by the two licensed HPVs vaccines, and found that the bivalent vaccine was more efficacious against 'high-risk' HPV 31, HPV 33 and HPV 45 compared to the quadrivalent vaccine (Malagón et al., 2012).

Molecular sequence analyses from the regions of America, Africa, Asia and Europe have shown a high degree of sequence conservation between isolates investigated for both HPV 6 and 11 genotypes (Ahmed et al., 2013; Danielewski et al., 2013). Findings of those analyses revealed that the genomic diversity of HPV 6 and 11 has a minimal effect on vaccine antibody formation for these genotypes (Ahmed et al., 2013). On the other hand, for HPV 16 and 18 genotypes, genomic diversity is high and the possibility of the presence of significant polymorphisms that can interrupt L1 antibody formation remain to be elucidated. For other 'high-risk' HPVs, not included in the current HPVs vaccines, such as genotypes HPV 31, 33 and 58, the genomic diversity is much higher, mostly mapped to surface-exposed domains and in some cases within known neutralizing antibody epitopes (Ahmed et al., 2013). These data highlight a number of variant amino acid residues that require further investigation for future vaccine development by covering more 'high-risk' HPVs and aiming to offer a higher protection rate against cervical cancer.

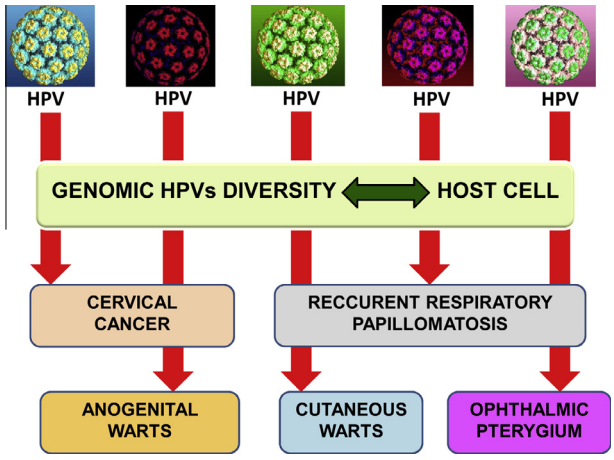

Fig. 2. Genomic HPVs diversity and host-cellular interactions as the key point of different clinical manifestations of HPVs infection in adulthood and childhood.

6. Future directions

Genomic HPVs diversity can interact with host-cellular mechanisms and this interaction can have a significant impact on the clinical course of different HPV-associated diseases, as presented in Fig. 2. Further analysis of the genomic diversity of HPVs can offer additional data on the natural history and pathogenesis of HPVs. The introduction of advanced molecular techniques, such as the next-generation sequencing technologies, in the laboratory will enable the development of more reliable HPV typing methods, allowing the clinicians to identify the exact oncogenic potential of different HPVs in each patient separately. This will have a significant impact on the prediction of the prognosis of HPVs infections and will definitely influence the treatment's choice. Moreover, these techniques may reveal, isolate and characterize novel HPVs variants. Furthermore, the next-generation sequencing could ideally be employed in multi-center studies to provide a rapid, sensitive and accurate solution for HPV monitoring and even for the effective vaccine development.

Epidemiological studies should be conducted to investigate the relationship between different HPVs variants and different phenotypes in order to clarify the exact clinical impact of different sequence variations. This will help in establishing a database concerning the genomic HPVs diversity and pathogenicity of different HPVs variants. Moreover, it can help clinicians to design and optimize diagnostics protocols in order to reduce HPV-associated diseases in both adulthood and childhood. It can also be used to establish efficient diagnostic tools to predict more accurately the prognosis of HPVs infections. Consequently, clinicians will gain a better understanding of the oncogenic potential of the virus in each case separately and will be able to improve the management of the patients. Such considerations might be facilitated by complete sequencing of variant capsid genes followed by the development of immunologic test systems.

In the future, an additional challenge will be to determine whether HPVs variants are relevant to vaccine strategies against HPVs. Single amino acid substitutions within the L1 and L2 capsid genes may be important in the viral escape from neutralizing antibodies. To achieve these conditions more studies are needed in order to find new broad-spectrum vaccines that are more cost-effective. The future multivalent VLP-based vaccines are expected to extend coverage to a wider array of HPVs genotypes, despite their genomic diversity. The future aim of eradicating HPV-associated pathologies worldwide may be achieved by locally producing antigens with cross-activity among the different types of HPVs. Given that specific HPVs variants play an important role in human cancer and variant lineages have different pathologic potentials, a comprehensive evolutionary study and classification system is

needed. However, the above-mentioned clinical benefits on adult and children populations are likely to emerge only when prevention strategies are harmonized and clear and complete information is disseminated.

Competing interests

The authors have declared that no competing interests exist.

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