CHAPTER II

LITERATURE REVIEWS

1. The HPV classification and genome organization

Human papillomaviruses (HPVs) are classified into *Papillomaviridae* family. The HPVs are small nonenveloped particles with icosahedral capsid of 52-55 nm in diameter. HPV genome is a double-stranded, circular DNA genome of approximately 8,000 base pairs (bp) in size. The capsid consists of two structural proteins, L1 and L2 proteins. The L1 is a major capsid protein which has a molecular weight approximately 55 kDa and represents for 80% of the viral particle. The L2 is a minor capsid protein which has a molecular weight approximately 70 kDa.

Figure 1 shows HPV genome which is divided into three major regions. The first is a ~1 kb non-coding upstream regulatory region known as long control region (LCR). It contains many of cis elements, which regulate viral replication and gene expression. The second region is a ~4 kb early (E) region encoding nonstructural proteins. The third region is a ~3 kb late (L) region comprising of ORFs L1 and L2 that encodes the L1 and L2 structural proteins for viral capsid.

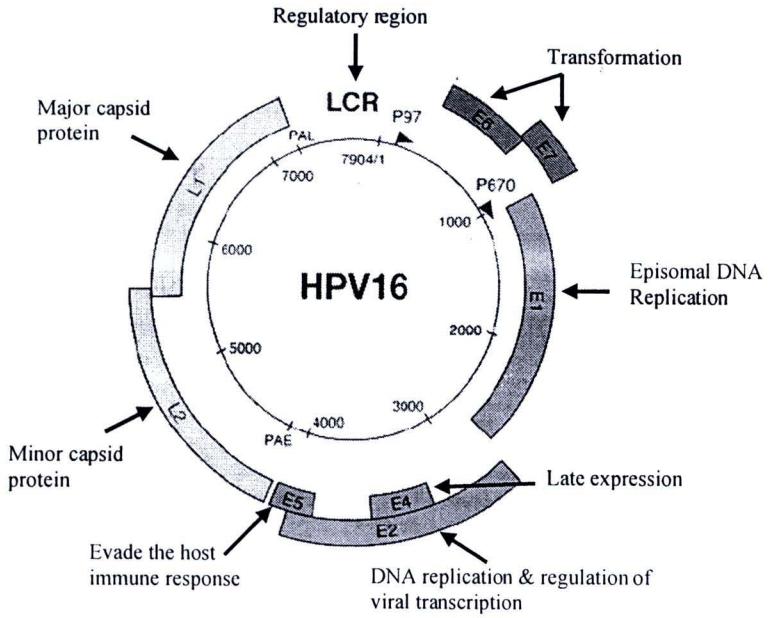

Figure 1 A schematic representation of the circular HPV 16 DNA genome organization modified from Doorbar, 2006 and Boulet, 2007 [17, 18].

Long Control Region (LCR) or Upstream Regulatory Region (URR)

The LCR or URR of HPV does not encode for any protein and is the less conserved region among papillomaviruses. It contains the early promoter and various transcriptional regulatory motifs including activator protein 1 (AP-1), AP-2, nuclear factor 1 (NF-1), glucocorticoid response elements (GRE), transcriptional enhancer factor 1 (TEF-1), Yin Yang-1 (YY-1), octamer-binding transcriptional factor-1 (OCT-1), *trans*-acting transcription factor-1 (SP-1), CCAAT displacement protein-1 (CDP)/Cut that overlaps the binding site of the E1 replication proteins and E2 binding sites [9-12, 19, 20]. The origin of replication (ori) is also located within the LCR, normally centered between two E2 binding sites.

Positive acting transcription factors are NF-1, AP-1, OCT-1, TEF-1, SP-1 and glucocorticoid response elements. Negative acting transcription factors are transcriptional factor YY-1 and CDP1/Cut [9-11]. The LCR of HPV regulates transcription from the early and late regions, furthermore, this region will control the viral protein production. Because the LCR contains many specific transcriptional binding sites, it may play a role in determining the host range of specific types of HPV [20].

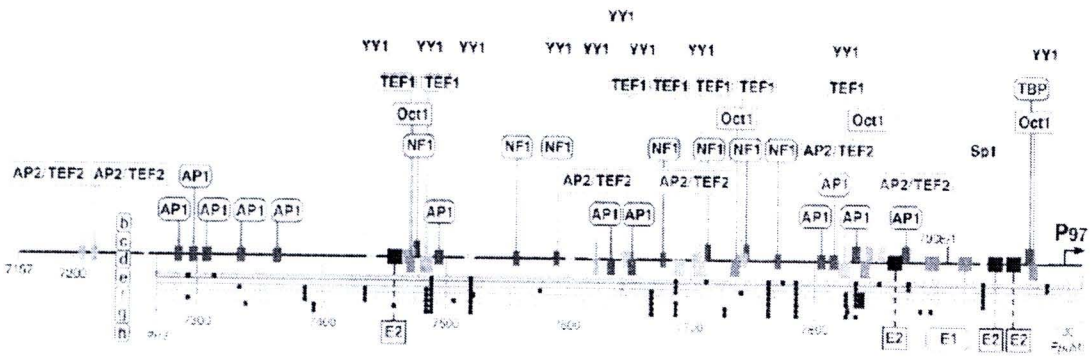

Figure 2 Schematic represents the linear map of LCR and specific transcriptional binding site genome organization of HPV 16 European prototype [12].

Early Region

The early region is downstream of the LCR. This region consists of 6 open reading frames (ORFs), E1, E2, E4, E5, E6 and E7 as shown in the Figure 1. ORFs are DNA segments that are transcriptional units and capable of encoding for proteins [20]. The early ORFs of HPV are encoding for early proteins that have the same name of each early ORF. The functions of early proteins are involved in the replication and transcription of HPV DNA, and transformation of infected cell because these segments will expressed in nonproductively infected cells and in transformed cells. The functions of each early protein are summarized in the Table 1.

Late Region

The late region is between early region and LCR. This region consists of 2 ORFs, L1 and L2 which encode for viral capsid proteins, as shown in the Figure 1. L1 ORF encodes for the major viral capsid proteins, which is highly conserved among difference papillomavirus species and containing 360 copies of the protein organized into 72 capsomers. L2 ORF encodes for the minor viral capsid proteins, and may present in the centre of pentavalent capsomers [17]. Both L1 and L2 viral capsid proteins are expressed in productively infected cells.

The summarize of HPV genome and protein function are shown in the table 1.

At present, more than 200 HPV genotypes have been classified according to their biological properties, oncogenic potential and phylogenetic position. About 40 HPV types are able to infect the mucosal genital tract that phylogenetically classified mostly in HPV Alpha genus. The Beta genus is associated with inapparent cutaneous infection in humans, cutaneous epidermodysplasia verruciformis and in patients melanoma skin cancer [17, 21].

Table 1 Functions of the products of HPV early and late region open reading frames and HPV non-coding upstream regulatory region (LCR) modified from Boulet, 2007 [18].

HPV region	Functions
LCR	Regulation of viral replication and transcriptional of HPV early region.
E1	Helicases fuction; Essential for viral replication that interacts with E2 protein, control of gene transcription and maintenance of viral episome.
E2	Viral transcription factor; Essential for viral replication, regulates viral DNA transcription and replication and repression of E6 and E7 proteins. Interaction with cytoskeleton proteins; viral assembly
E4	Late expression: Play a role in productive infection, allowing release of virus-like particles.
E5	Helping the virus evade the host immune response, Interfere with antigen presentation by prevents transport of the MHC I; HLA class I in humans to the cell surface.
E6	Cell immortalization; p53-degradation; telomerase activation; anti-apoptotic effect; induction of genomic instability
E7	Cell immortalization and transformation of host cell; interaction with pRb and pRb-associated pocket proteins; transactivation of E2F-dependent promoters; induction of genomic instability
L1	Major capsid protein.
L2	Minor capsid protein; role in recruiting viral genomes for encapsidation; involvement in nuclear transport of viral DNA

The classification of HPV genotypes is based on nucleotide and amino acid sequence alignments. Different HPV genotypes are defined as having more than 10% variation in specified regions of the genome, E6, E7 and L1 ORFs. New HPV

genotypes have to be registered with the Reference Center for Papillomaviruses at the German Cancer Research Center in Heidelberg to confirm.

The term "subtype" is used to identify isolates of HPV types with divergent restriction patterns. Subsequently, this term became redefined as referring to an isolate whose L1 sequence is 2-10% different from that of known HPV type. Nowadays only three HPV isolates are known that full fill this subtype definition. HPV 46, HPV 55 and HPV 64 had been originally described as separate types, but their type status has now been cancelled, as they are subtypes of HPV20, HPV44 and HPV34, respectively [21].

The term " variants or intratypic variants" is used to classify HPV types, when they show sequence differing less than 2% in the coding region and 5% in the non coding region compared to the prototype [22, 23].

HPVs infection is associated with a variety of clinical conditions that are vary from innocuous lesion to cervical cancer. The difference of HPV type infections and disease associations are shown in the Table 2. HPVs are divided into two groups as high-risk (HR) and low-risk (LR) HPV types according to their potential to induce malignant transformation. Based on pooled data from 11 case-control studies of the association between cervical cancer and HPV infection from multiple countries[5], 12 HPV types have been classified as high risk for development of cervical cancer, 6 have been classified as probable high risk and 12 have been classified as low risk causing genital warts (condyloma acuminata) as showed in Table 3.

2. HPV life cycle

HPV life cycle is linked to the differentiation program of the infected host cells. HPV infection occurs in the basal cell layer by infectious particles through microwound of the epithelium. The viral particles are bound to basal cell surface receptors that allow initial attachment of the virus to the cell. Previous studies have suggested a dependence on the presence of heparin sulphate [24, 25]. Internalization of bound virions occurs through the endocytosis of clathrin coated vesicles [26, 27].

Table 2 HPV types and diseases association [1].

Diseases	HPV types
Plantar warts	1, 2, 4, 63
Common warts	2, 1, 7, 4, 26, 27, 29, 41, 57, 65, 77, 1, 3, 4, 10, 28
Flat warts	3, 10, 26, 27, 28, 38, 41, 49, 75, 76
Other cutaneous lesions (e.g., epidermoid cysts, laryngeal carcinoma)	6, 11, 16, 30, 33, 36, 37, 38, 41, 48, 60, 72, 73
Epidermodysplasia verruciformis	2, 3, 10, 5, 8, 9, 12, 14, 15, 17, 19, 20, 21, 22, 23, 24, 25, 36, 37, 38, 47, 50
Recurrent respiratory papillomatosis	6, 11
Focal epithelial hyperplasia of Heck	13, 32
Conjunctival papillomas/ carcinomas	6, 11, 16
Condyloma acuminata (genital warts)	6, 11, 30, 42, 43, 45, 51, 54, 55, 70
Cervical intraepithelial neoplasia (CIN)	
- Unspecified CIN	30, 34, 39, 40, 53, 57, 59, 61, 62, 64, 66, 67, 68, 69
- Low-grade CIN	6, 11, 16, 18, 31, 33, 35, 42, 43, 43,44, 45, 51, 52, 74
- High-grade CIN	16, 18, 6, 11, 31, 34, 33, 35, 39, 42, 44, 45, 51, 52, 56, 58, 66
Cervical carcinoma	16, 18, 31, 45, 33, 35, 39, 51, 52, 56, 58, 66, 68, 70

Table 3 Epidemiological classification of HPV types [5].

Group	HPV types
Established high risk	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59,
Probable high risk	26, 53, 66, 68, 73, 82
Established low risk	6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, CP6108

Uncoated virus may be facilitated by the disruption of intracapsomeric disulphide bonds in the reducing environment of the cell allowing viral DNA to be transported to the nucleus [28]. In the nucleus, the virus maintains its genome as a low copy number as approximately 50-100 copies per cell [1] throughout the course of infection. During viral DNA replication, the viral E1 and E2 proteins are expressed. The binding of E2 to the HPV LCR is necessary for viral DNA replication, and recruits the E1 DNA helicase to the viral origin of replication [29]. Both proteins form complex to bind to sequence at the origin of viral replication and recruit cellular polymerase and accessory proteins to mediate replication [30]. Nonproductive stage of the HPV life cycle occurs in the terminally differentiated layers of epithelium in which the late genes are expressed and virions are produced [31] . As HPV infected basal cells differentiate and migrate to surface, they remain active in the cell cycle due to the action of E7 proteins [32]. E6 and E7 proteins interact with cellular proteins. These interactions have been shown to induce proliferation and eventually immortalization and malignant transformation of cells [33]. E4 and E5 are viral early genes product that may involved in the regulation of late viral function. In productive cycle, the assembly of infectious particle occurs by the expression of capsid proteins L1 and L2 in the upper layers of the epithelium. Following virion assembly, mature viruses are released. The HPV life cycle is shown in the Figure 3.

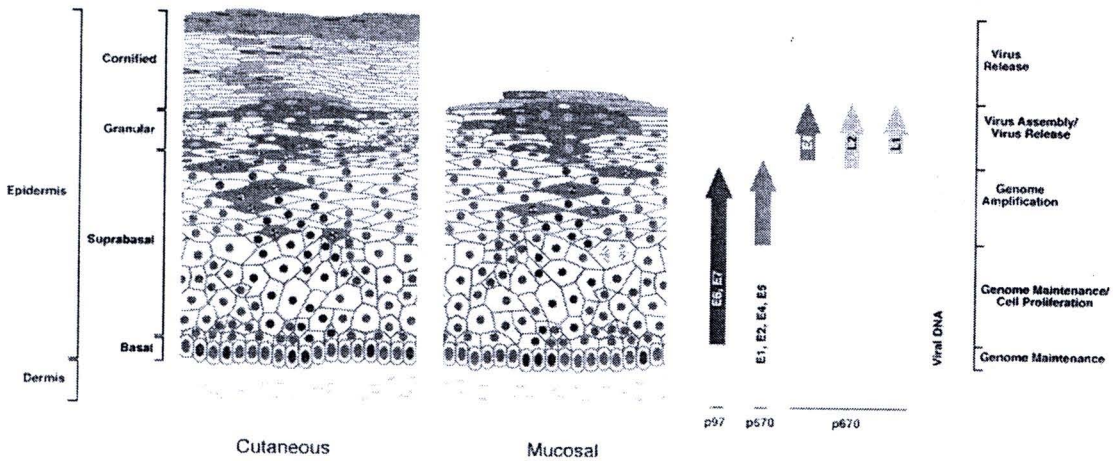

Figure 3 The HPV life cycle [17]. The key events that occur following infection are shown diagrammatically on the left. The epidermis and underlying dermis are shown. The different cell layers presenting in epi-thelium are indicated on the left. Cells in the epidermis expressing cell cycle markers are shown with red nuclei. The appearance of such cells above the basal layer is a consequence of virus infection, and in particular, the expression of the viral oncogenes, E6 and E7. The expression of viral proteins necessary for genome replication occurs in cells expressing E6 and E7 following activation of p670 in the upper epithelial layers (cells shown in green with red nuclei). The L1 and L2 genes (yellow) are expressed in a subset of the cells that contain amplified viral DNA in the upper epithelial

3. HPV and cervical cancer

The causative link between HPV and cervical cancer has become evident from epidemiological and functional study. HR HPV infection has been implicated as a primary aetiologic of cervical cancer because more than 99.7 % of cervical cancers contain HR HPV DNA [17] and HPV 16 is the most prevalent type. In the development of cervical cancer, the integration of HR HPV genome into host genome or chromosome are the key events of HPV-induced carcinogenesis. These evidence are subsequent consistently in the overexpression of E6 and E7 viral oncogene, whereas other portions of viral DNA may be deleted or disturbed. E6 and E7 viral

oncogene expression may result in the immortalization of infected cell and progression to preinvasive lesion to cervical cancer in finally [34].

E6 oncogene

E6 oncoprotein has a molecular weight approximately 17 kDa in size and contains approximately 150 amino acids and 2 zinc finger-like motifs [35]. It plays an important role in the tumorigenecity of HPV infection. The HR HPV E6 proteins are localized to both the nucleus and cytoplasm of the host cell. One of the functions of HR HPV E6 protein is its ability to interfere with p53-mediated cell cycle regulation.

p53 is a tumor suppressor protein that induces cell cycle arrest or apoptosis because it involves in regulation of G1/S and G2/M cell cycle checkpoints. E6 binds to p53 with an ubiquin ligase called E6-associated protein (E6AP), which results in the ubiquitination of p53 and its subsequent proteolytic degradation.

Binding of PSD-95/dise large/ZO-1 (PDZ) domain and activation of human telomerase reverse transcriptase (hTERT) expression are two important p53-independent way for E6 proteins to immortalize human cells [36]. PDZ proteins are involved in a variety of functions including cell signalling and cell–cell adhesion [37]. The association of E6 with PDZ proteins appears to play a major role in the HPV life cycle as human foreskin keratinocytes (HFKs) transfected with HPV 31 genomes containing mutations in the E6 PDZ-binding domain display reduced growth, as well as a reduction in early transcription and episomal maintenance [38]. In transgenic mice expressing HPV 16 E6, the ability to bind to PDZ proteins was shown to be required for the induction of epithelial hyperplasia.

hTERT is the catalytic subunit of the enzyme telomerase. HR HPV E6 proteins have been shown to increase telomeric length in epithelial cells by activating the Htert [36, 39] leading to immortalization of human cells.

E7 oncogene

E7 oncoprotein has a molecular weight approximately 12 kDa and contains approximately 98 amino acids incorporate three conserved regions called CR1, CR2 and CR3. The CR1 domain comprises the amino terminus, CR2 contains an LXCXE motif that mediates binding of E7 to retinoblastoma (Rb) tumour suppressor protein family members, and CR3 consists of two zinc finger motifs. E7 also contains a casein kinase II phosphorylation site that leads to phosphorylation of E7 during the

G1 and S phases of the cell cycle[35]. The critical role of HPV E7 protein is its contribution to transformation of cells through its affects on Rb family. Rb protein is an inhibitor of cell cycle progression from the G1 to the S phase. In HPV-infected cells, E7 proteins can override the function of normal cell cycle control through binding and degradation of hypophorylated Rb via a proteosome-dependent pathway. This leads to releasing of E2F complexes and result in permits cell cycle progression to S phase that allows DNA replication.

Furthermore E7 protein can disrupt the Rb-HDAC complex leading to S phase entry of cell cycle. HDAC is histone deacetylase that play a role in cell cycle regulation. It is recruited to inhibit E2F-regulated S phase specific gene by hypophosphorylated form of Rb protein. The activation of E2F by E7 proteins in differentiated epithelia is suggested to be a common mechanism for viral replication in both HR and LR HPVs [40].

4. HPV infection and other co-factors of cervical cancer development

Transmission of HPVs occurs primarily through skin-to-skin contract. Epidemiologic studies suggest that sexual activity is influenced for the risk of genital HPVs infection and associated with cervical cancer. Sexual activity at an early age and multiple sex partners appears to be higher risk for contracting genital HPVs. HPV infection is common in sexually active young women, 18 to 30 years of age but cervical cancer is more common found in women older than 35 years suggest that HPV latent infections in a active young women resolve spontaneously over 5 years, but, in a small fraction of women, persistent infection with high-risk HPV results in cervical intraepithelial neoplasia (CIN) grade 2/3 and eventually cervical cancer [41].

Many factors are thought to associate with development of cervical cancer. Infection with HR-HPV type is certainly the most common but insufficient for progression to carcinogenesis. Moreover, it is also quite known that some patients with HPV infection will clear up spontaneously, while others will progress to high grade lesions and finally will progress to cervical cancer. Previous studies suggested that potential cofactors associated with cervical cancer can be divided into three groups:

(i) Environmental factor, including hormonal contraceptive, smoking, parity and co-infection with other sexually transmitted agents such as long term use of oral contraceptive is a risk of invasive cervical cancer.

Studies from Adam et al and Brisson et al suggested that LCR of HPV contains sequences similar to the glucocorticoid responsive elements (GRE) that are inducible by steroid hormones such as progesterone (the active component of oral contraceptives) and dexamethasone [42, 43]. Therefore, HPV LCR, that are inducible by steroid hormones such as estrogen or progesterone result for enhancing HPV gene expression in the cervix via progesterone-receptor mechanism.

Possible mechanism of smoking induced to carcinogenesis is local immune suppression from mutagenic activity of cigarette components. These effects contribute HPV persistent infection, direct genetic damage and cervical cell malignant transformation similar to seen in the lung. Multiple pregnancies is associated with independent risk factor of invasive cervical carcinomas after adjustment for the number of sexual partner and the age at first for sexual activity.

Co-infections with other sexually transmitted agents may serve as a cofactor in development of cervical carcinogenesis such as the co-infections with HSV-2, *Chlamydia trachomatis* and HIV. A multi-case control study by Smith JS et al, [44] suggested that HSV-2 positive case was associated with an increased risk of both squamous cervical cancer and adeno squamous carcinoma (ADSC) among HPV-positive women with 2-fold increased risk for cervical cancer.

(ii) Viral cofactor such as HR-HPV type infection, co-infection with other HPV types, HPV variant, viral load and viral intregration. It has been suggested that infection of HR-HPV type is capable to induce invasive cervical carcinoma even when they are present at low levels.

HPV variant is the one factor associated with development of cervical cancer. HPV 16 is the most common studies on HPV variant .based on sequence variation of L1, L2, E6, E7 and LCR region [4]. HPV 16 variant can be divided into five major groups; European (E) branch as prototype and European variant, Asian (As), Asian–American (AA), African-1,2 (Af-1,Af-2) and North-American variant (NA-1). Many previous studies suggested that infection with HPV 16 variant was the increased risk factor to cervical neoplasia more than infection with HPV 16 prototype [14, 16, 45].

Co-infection with multiple HPV types has been reported. The presence of multiple HPV genotype infection tends to increase with the severity of cervical disease.

(iii) Host cofactor, including genetic factor and factor related to the immune response. Genetic variation between individuals in immune-related genes might be of interest because only a small fraction of HPV infected women is developed to cervical cancer. To date, Human leukocyte antigens (HLAs) have been the most studied.

5. HPV 16 prevalence

The prevalence of cervical infection with HPV in women varies greatly worldwide, and is closely related to the corresponding risk of cervical cancer. Epidemiologic studies have demonstrated strong and consistented associations between detection of HPV 16 DNA and risk for CIN and cervical cancer [46, 47]. HPV 16 is the most common HPV type that found in nearly all countries of the world. The study about HPV types in invasive cervical cancer from a pooled analysis of 12 studies conducted in 25 countries has estimated HPV type-specific prevalence in 3085 cervical cancer cases. The most common prevalence HPV type is HPV 16, which found more than 50% as shown in the Figure 4.

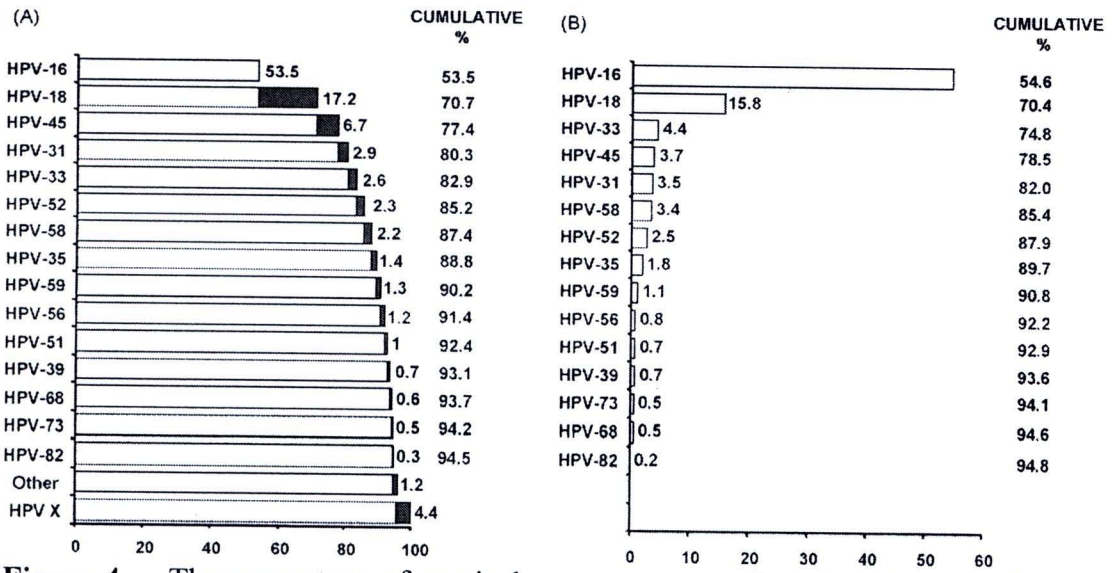

Figure 4 The percentage of cervical cancer cases attributed to the most frequent HPV types in all regions combined, as estimated from pooled analysis of HPV type-specific prevalence in 3085 cervical cancer cases and a meta-analysis of more than 14,500 cases [48].

Moreover, many studies on HPV 16 prevalence and distribution in Asia were reported. In Taiwan, studies on type-specific distribution of HPV in relation to grades of cervical neoplasia by Huang *et al* [47] suggested that samples were HPV DNA positive for 96.3% and HPV 16 was found the most common and detected in 37.2% of LSIL, 50.5% of HSIL, and 65.9% of ICC, respectively. The detection rate of HPV 16 significantly increased with the severity of the cervical lesions ($P < 0.0001$). In India, Sowjanya *et al*,[49] studied on HR-HPV prevalence and distribution in invasive squamous cell carcinoma of the cervix. They suggested that among the HPV positive cancers, the overall type distribution of the major high-risk HPV types was as follows: HPV 16 (66.7%), HPV 18 (19.4%), HPV 33 (5.6%), HPV 35 (5.6%), HPV 45 (5.6%), HPV 52 (2.8%), HPV 58 (2.8%), HPV 59 (2.8%) and HPV 73 (2.8%). The study regarding to common HPV subtype in Pakistani women with cervical carcinoma found that 94.92% of HPV positive samples were HPV 16 [50].

In Thailand, prevalence of HPV 16 in Thai woman population is reported.The study of case-control study by Chichareon *et al.* suggested that the most common HPV type in women with squamous cell carcinoma was HPV type 16. Study by Chopjit *et al*,[16] in Northeast Thailand on prevalence of HPV 16 and its variants in abnormal squamous cervical cells found that HPV 16 was present in 16.7% (9/54) of normal cervical cells, 38.9% (14/36) of CIN II-III, and 75% (30/40) of squamous cell carcinomas, corresponded to an associated risk of cervical cancer (OR 22.370; 95% CI 9.952-50.283). HPV 16 was significantly associated with abnormal tissue and squamous cell carcinoma.

6. HPV 16 variant and HPV 16 As variant

HPVs are the large diverse group of DNA viruses involved in human disease. According to the evidence of HPVs infection and association with cervical carcinogenesis, one of viral factors that involved is the role of HPV sequence variation or HPV variants. Previous studies on HPV variants in squamous neoplasia of the cervix suggested that different HPV variants have altered biochemical and biological properties and represented an additional risk factor in the development of squamous intraepithelial lesions and invasive carcinoma of the cervix [51]. Given the most prevalence of HPV 16 infection (more than 50%) in women with cervical cancer

case worldwide, HPV 16 variant is the most mainly extensive studied. Sequence analysis of HPV 16 E6, L1, L2 and LCR, HPV 16 variants have been classified and grouped into six naturally distinct phylogenetic brances: European (E), Asian (As), Asian-American (AA), African-1 (Af-1), African-2 (Af-2) and North American (NA) [4, 15, 52]. However, intratypic sequence variation has also been found in the E2, E4, E5 and E7 genes of HPV 16 [1]. Previous studies suggested that HPV 16 As variant was found predominantly in Asia and it is rare or absent in other continents. Studies of the HPV 16 variant in geographical distribution based on E6 hybridization patterns in 408 invasive cervical specimens found 26% of Southeast Asia specimens were As variants [4]. The E6 sequence signature patterns used for identification of phylogenetic classes of HPV 16 As variant are nucleotide change in the position ~ 178 T > G when compare with the prototype HPV 16 reference sequence [4]. Moreover, studies in China reported that the high prevalence of HPV 16 variant is HPV 16 As variant and might contribute to the high incidence of cervical cancer in China [13, 15]. Study on HPV 16 variant in Khon Kean population, Thailand by Chopjitt *et al* suggested that the most common variant found in Thai women was HPV 16 As variant (58%) and showed a risk association in 73.9% of SCC and 57.1% of CIN II-II [16]. HPV 16 variant worldwide distributions are shown in the figure 5.

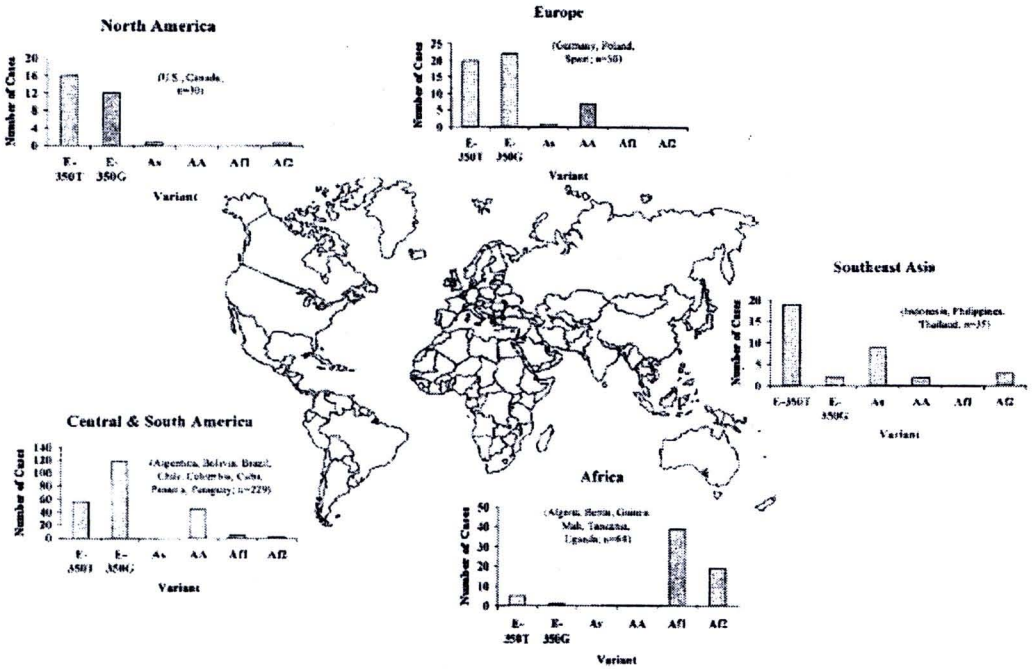

Figure 5 Geographical distribution of HPV 16 variants worldwide.[4, 51]

7. HPV 16 LCR polymorphism and transcriptional activity

HPV 16 LCR is a genomic segment, without any major open reading frames but contains a large number of cis-responsive elements that provide for viral gene expression and replication. LCR sizes approximately 850 bp in the case of genital HPVs [53] and locates between the end of the L1 gene and the start of E6 gene. HPV 16 LCR divided into 3 regions (5′ segment, central segment, and 3′ segment). Many specific protein binding sites of both viral protein and host protein are located in the LCR as described above. Especially, E6 and E7 oncogenes, are expressed directly by the transcriptional promoter P97 located at the E6-proximal end of the LCR [9]. Therefore, nucleotide sequence variation in the LCR may leads to differences in the binding affinity of some specific proteins of viral and host protein, which have an impact to enhancement of E6 and E7 oncogenes expression. Previous data suggested that the LCR has been found the most variable region and nucleotide variability within the LCR could lead to enhance the oncogenicity of this virus. Nucleotide sequence variation of the LCR can be used to classify HPV 16 variants as described above.

Many studies of HPV 16 LCR sequence variation suggested that HPV 16 LCR sequence variation at position ~ 7840 - ~7842 (G>A) were found only in Asian patients and used to classify for HPV 16 As variant [54-56]. Previous data reported the high frequencies of HPV 16 LCR nucleotide sequence variation are at position 7193(G>T) and 7521(G>A), which are TEF-1 and YY-1 binding site, respectively. These mutations have been commonly found in the patients with cervical cancer worldwide [9, 23, 57]. study of O'Conner *et al* on the HPV 16 LCR mutation overlapping YY-1 binding sites, which are more commonly detected in cancer samples have showed promoter activity higher than prototype [11]. Chen *et al* studied on mutation in the LCR and their functional consequences in oral cancer in 1997. They found that promoter activity of the mutated HPV 16 LCR was significantly higher than wide type. They also suggested that mutations in the LCR of HPVs in oral cancer could lead to the increased expression of E6/E7 oncoproteins, which might contribute to the carcinogenic process [19]. Sichero *et al* studied on functional implication of HPV gene variability and summarized that variation in the LCR resulting in (i) change in binding affinity of different cellular transcriptional factors (ii) creation or elimination of transcriptional factors-binding sites (iii) change in transcriptional activity [58].

LCR functional analysis with transcriptional activity is important to evaluate the association between the transcriptional activity of LCR and oncogenic potential of HPV 16 variants. Several studies have suggested that the sequence variation in LCR of HPV 16 may modulate the oncogenic potential of HPV 16. Especially, sequence variation in the part of enhancer regions of LCR, which contains several specific protein binding sites such as transcriptional regulator proteins YY-1, AP-1 and NF-1 binding sites, were found associated with the risk for progression of cervical cancer by enhancing the expression of E6 and E7 viral oncogenes [9, 10, 12, 59].

Park *et al* studied on mutation in the HPV 16 LCR derived from Korean women and LCR functional analysis using CAT assay found that HPV 16 LCR mutation at YY-1 binding site increased the promoter activity and contributed to the progression of cervical cancer in Korean women [55].

Tornesello *et al* studied on functional analysis of sequence rearrangements in the LCR of HPV 16 Af-1 variants isolated from Ugandan penile carcinomas in 2000.

They found that HPV 16 LCR rearrangements were frequently found in African penile carcinomas biopsies, which might increased HPV 16 LCR activity and associated with an increased E6/E7-mediated in vitro transforming activity [60].