JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Phylogeny of the Human papillomavirus: a study based on viral genes

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Abstract:

Human papillomavirus (HPV) is the virus that contributes the highest to oncogenic transformation and consequently cancers. HPV is of high and low-risk types. This shows that HPV is a highly evolved virus. The key to combatting this virus lies in learning its evolutionary patterns and hence the sites that can be used as molecular targets. This study is in concurrence with studies so far and shows evolutionary changes in the L1 gene and conservation in the E6 and E7 genes.

Keywords: evolution, HPV, phylogeny, E6 gene, E7 gene, L1 gene.

INTRODUCTION

Viruses are intercellular parasites whose classification is often confused between living and non-living. Viruses to thrive viruses need a host. Viruses manipulate the host mechanism by insertion of their genetic material and using the host as a source of energy, synthesis of survival macromolecules, replication and assembly of the genome, and consequently the establishment of the virus inside a host cell (25). Viruses for survival and infection in the host cells have a cascade of mechanisms. These mechanisms constantly evolve to overcome the host defense mechanisms. The viral life cycle is divided into 6 major parts from the attachment to the host to viral replication and propagation in the host. Shortly these steps are attachment to the host, penetration of the cell membrane, uncoating of the virus, gene expression with integration to host genetic material, replication through the lytic and lysogenic cycle, assemble of the virions, and finally viral release and increase in viral load (18). Viruses can be defined simply as enormously plentiful, highly genetically varied, quickly evolving entities. Viruses evolve to form antibody escape mutants and some stains get filtered out while others thrive and circulate in the population. The fascinating interest in viral evolution has stemmed in the latest times with the rapidly evolving coronavirus.

Among the DNA Viruses, the HPV virus is known to cause most genital warts and accounts for the major portion (90-95%) of the total cervical cancers.

The origin of viruses has forever remained an intriguing subject of study. The methods of evolution have been proposed so far in this regard 1. Virus –first approach.2. Escape hypothesis 3. Reduction hypothesis.

These approaches are explained as 1. Virus first approach –is the approach that states that at the beginning of time, there existed no cellular forms but just RNA molecules and enzymes capable of self-replication. 2. Escape Hypothesis-viruses states that viruses are formed from RNA /DNA fragments for plasmids or transposons and during fission reaction, smaller molecules can be engulfed and a self-replicating RNA segment could have formed a virus. Finally, 3. The reduction hypothesis postulates that viruses have originated from primordial cells that have lost other elements except for genetic material (12).

Mechanisms of Viral evolution

All living beings evolve in an attempt to adapt to the changing environment. Each organism undertakes different mechanisms to adapt. Viruses being very small either evolve on the coat protein or the genetic level hence having a wide variety of viruses. Mechanisms that a virus uses for evolution are 1. single Nucleotide changes 2. Recombination and horizontal gene transfer 3. Tandem repeat fluctuations and 4. Insertions, deletions, and duplications. Most of these evolutionary changes take place due to host and virus interactions. Depending on the host and viral interactions mutations occur. These genetic changes are the key point of the evolution of a virus. Some factors that affect the evolution of a DNA virus are – the structure of the host cell, periods of infection, host cell intricacies, acute/persistent infection, evolution/divergence with the host, and finally natural selection and checkpoints for survival. Viruses follow a cell-based life type evolution but tend to evolve rapidly. When two viruses infect a cell/ individual a crossover takes place to form a new mixed virus. However, RNA viruses are known to have a higher rate of evolution and drug resistance. One major part of viral evolution is the large the population size, the larger the population more the chances of mutation. In the case of Influenza, studies show two models – antigenic shift and antibody drift.

Viruses are classified by several means based on genome structure and core, capsid structure, and the newest classification –The Baltimore classification. Based on genome structure and core the viruses are classified as RNA, DNA, Single-Stranded, Double-stranded, Linear, Circular, Non-segmental, and Segmental. Classification based on capsid structure – Naked icosahedral, enveloped icosahedral, Naked helical, Enveloped helical, Complex with protein icosahedral and complexes. Baltimore classification groups viruses according to mRNA produced during the replication of the virus. Single-stranded DNA, Double-stranded RNA, Single-stranded RNA –positive strand, Single-stranded RNA – negative strand, Single-stranded RNA with reverse transcriptase, and double-stranded DNA viruses with reverse transcriptase (19).

Human Papilloma Viruses

DNA viruses of small, non-enveloped viruses of 52-55nm with an icosahedral structure are papillomaviruses. A single double-stranded DNA virus with 8000 base pairs encapsulated in a 72 pentameric protein capsid capsomer while bound to histones is the brief structure of the HPV virus. HPV virus is classified into the papillomaviridae family. Currently, this family contains 30 genera formed by 189 types of papillomavirus (7). This virus is isolated from different types of species humans, non–human mammals, birds, and reptiles. HPV is of 5 genera. The variants are recognized by the L1 (late) gene (1). A variation in the L1 gene when the whole genome is cloned validates it as a variant (1% variation), subtype (2-10 % variation), and a new HPV virus for variations more than 10%.(23).

Alpha papillomaviruses consist of a group of viruses that are both low-risk and high-risk HPV viruses, that cause mucosal and cutaneous lesions in humans and primates. High-risk HPV are those that cause pre and malignant lesions by causing neoplastic changes in the keratinocytes (6). Low-risk HPV causes benign lesions. HPV 16, 18, 45,32,10,61,2,26,53,7,34,1, 54, etc belong to the group alphaviruses. HPV is classified into high-risk and low-risk based on the nature of the infection. HPV is classified as both mucosal and cutaneous group. The division into high and low-risk groups is based on the capacity of the virus to cause cancer. Low-risk mucosal HPVs produce genital warts, however, high-risk HPVs produce squamous epithelial lesions and can cause invasive squamous carcinoma, such as cervical cancer. Beta –papillomavirus such as 5,9,49 are responsible for cutaneous lesions and are often latent infections in the case of immune suppression. Gamma-papilloma viruses also form cutaneous lesions and have a distinguishable property by producing intra-cytoplasmic inclusion bodies. Mu and Nu papillomaviruses also cause cutaneous lesions with the difference that nu causes malignant lesions ((9). From studies, it is seen that the most prevalent of the HPV types is HPV 16 which causes most genital cancers. In this study, the emphasis is on the HPV viruses and their phylogenetic evolution.

Mechanism of infection of Human papillomavirus

Papillomaviruses begin their infection from epithelial cells, this is followed by the cause of micro-abrasions in the outer epithelium and entry of the virions. These virions form heparin sulfate proteoglycan complexes on the basement membrane. The genes of the HPV e1-e7 help in the transformation process and the consequent replication of the virions and release into the host. Meanwhile, the L1 and L2 proteins undergo conformational changes and help establish an infection (21).

HPV proteins and their role in infection

HPV viruses are viruses of approximately 8 kilobase pairs of double-stranded non-enveloped DNA. They mainly encode for 8 proteins 6 ‘ early ‘ proteins and 2 ‘late ‘ proteins. The early proteins have regulatory functions. The HPV life cycle begins with E4 followed by the L1 and L2 genes. The E1, E2, and E5 proteins usually function and help with the genome transcription and replication and are often expressed throughout the cycle, sometimes in lesser amounts at the later stage of infection. The main proteins in the infection pathway are the E6 and E7 genes. They help with blocking apoptotic pathways and promoting cell survival. L1 and L2 are the genes expressed after the E4 gene they help in transmission, spread, and survival. Some wide functions of the proteins are:-

The E1 protein of the HPV genome is the only protein with enzymatic activity. The major role in the viral life cycle is to help with genome replication. This protein works with the ATP-dependent DNA helicases to help the virus to survive. This protein is seen overexpressed at the time of infection and helps with proliferation, and differentiation, and aids in the downregulation of immune response genes (3).

The E2 protein of the HPV genome also helps with transcription and additionally with replication, transcription, segregation, and encapsidation. It plays a key role in the life cycle of HPV. Often expressed at both the vegetative state and post-transcriptional stages of the life cycle. It helps with the regulation of gene expression. It helps with the sustenance of the cell cycle and apoptosis regulation (2). However E2 proteins have a short life span and often their expression is increased with the presence of other genes such as E1. E2 activates apoptosis in both HPV and transformed cells through the activation of caspase 8 (20).

The E4 protein is the first expressed protein in the HPV life cycle. The main functions of the HPV E4 protein are the remodeling of the cytokeratin network. It helps with the arresting of normal cell cycling and aids with the virion assembly. E4 protein level expression is a perfect marker of the levels of HPV expression (26).E4 protein expression marks the viral replication commencement (24). E4 helps in genome amplification.

E5, E6, and E7 are the main viral oncoproteins.

E5 protein of the HPV virus primarily controls cell growth, and differentiation and helps with immune modulation.E5 are short membrane proteins with ORFs that are expressed during the early viral life cycle (11).E5 impairs CD95L and helps to stop trail mediated apoptosis. This is the mechanism of early-stage viral apoptosis prevention. The E5 proteins bind to the ATPase enzyme and affect MHC class 1 expression and thus hampers cell–cell communication (15). E5 proteins act on EGF receptor, V- ATPase, MHC class 1, and cell-to-cell communication. The E5 protein of HPV plays important roles in tumorigenesis, and modulation of signaling pathways and hence can be a target for therapy.

The E6 protein of HPV protein is the main oncoprotein. It inhibits apoptosis and cell differentiation. It regulates cell shape, polarity, mobility, and signaling.E6 proteins inhibit apoptosis through proteolytic in activation of pro-apoptotic proteins such as p53, FADD, and pro caspase8 .E6 protein's role is in the process of transformation .E6 proteins help immortalize the keratinocytes and increase the life span. The E 6 proteins consist of 150 amino acids with 4c-x-x- c motifs. E6 protein's main function is the blocking of the p53 activity and hence promoting the immortalization (16) .E6 prevents the p 53 activity by induction of telomerase activity and makes transformation independent of p53. The mechanism for cellular transformation is the degradation by ubiquitination and prevents negative growth receptors. Caspase 8 is blocked by the E6 protein thereby preventing cell death. The death domain FAS and FADD are also blocked by p 53 with TNFα (13). Aids with loss of cell polarity by interacting with Tyk2, IRF3, NFX1/2/3, etc. E6 also helps with invasion by interaction with focal adhesion protein paxillin and extracellular matrix protein fibulin and favors cell growth and provides invasion. E6 also destabilizes and degrades other proteins such as TIPO.

E7 proteins are of approximately 100 amino acids made of acidic polypeptides. E7 protein plays a role in cell cycle control and controls centrosome duplication .E7 protein blocks the pRb gene and hence helps with immortalization. The E7 gene also activates the jak stat pathway signaling pathway.E7 proteins help with DNA transcription. E7 proteins help with the inhibition of apoptosis transformation through p600. Leads to chromosomal instability through TUBG2 (10). E7 also helps with nucleic acid synthesis proliferation through M2. E7 acts on ATM and ATR as a response to DNA damage response (22). E7 plays a major role in the viral life cycle and carcinogenic transformation. E7 also upsets the link between cellular differentiation and proliferation in normal epithelium which encourages viral replication.

L1 is the late protein present in the major capsid. L1 protein is made of 72 pentamers called capsomers and weighs about 55-kilo daltons This is the protein that helps with the formation of infection.L1 protein is the one where variations take place among the variants and subgroups. The L1 protein is a pentameric assembly of the viral shell. The HPV virus-like particles self-assemble in vitro through the major capsid L1. The virus-like proteins (VLP's) on the exterior surface are similar and hence indistinguishable from the native non–enveloped papillomavirus virion. L1 allows the selective uptake of viral genomic DNA into the viral lumen (5).L1 is translated from RNA species that begin with shared upstream regulatory regions (URR). The N- and c- terminals of L1 follow an extended invading arm-like pattern and form a floor between the capsomer bond (These factors are the reason L1 protein is an important target for vaccine production. After the entry of the virus, the viral particle binds to the basal membrane, the mature virus binds to the outer surface of the host cell through the L1 protein.

L2 is the minor capsid protein that helps with the uptake of L1.L2 plays a major role in viral assembly and infection process. L2 is a nuclear protein (14). However, L2 cannot for VLP's.L2 enables encapsidation of the circular and nucleosome bound viral genome assembly of the no –enveloped virions. L2 has 2 major functions 1. interaction with β –actin and t snare syntaxin 18. 2. Escape of viral genome from endocytic compartment after viral uncoating. Cleavage of the furin at the L2 site is an essential requirement for infection.

This study aims at looking at the phylogenetic analysis of HPV low and high risk from the active viral proteins. 12 types of HPV including HPV16, HPV 18, HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58 and HPV59 are classified as high risk .While some of the common types of low-risk HPV are 6, 11, 42, 43, 44, 54, 61, 70, 72, and 81 (17).

Materials and Methods

The sequences for the study were obtained from GEN Bank. Software tools - Clustal W and Clustal omega were used for the MSA and phylogenetic tree analysis- phylograms are used to represent the data.

Results

A phylogram is a branching diagram (tree) that is an assumed estimate of phylogeny. The branch lengths are proportional to the amount of inferred evolutionary change. This study aims to see the sequence similarities of HPV viruses to obtain common primer target regions, and also to see the evolution of the viruses based on their viral genes. Sequences from the GenBank were blasted to see sequence similarity followed by MSA and finally, a phylogenetic tree was generated.

High-risk HPV

The main viral oncogene which from the literature survey showed the highest persistent expression throughout the infection was taken for study.

ENTRY	ENTRY NAME	PROTEIN NAME	GENE NAME	ORGANISM	LENGTH
P06463	VE6_HPV18	Protein E6	E6	HPV18	158AA
P03129	VE7_HPV16	Protein E7	E7	HPV16	98AA
P17387	VE7_HPV31	Protein E7	E7	HPV31	98AA
P24835	VE6_HPV39	Protein E6	E6	HPV39	158AA
P54667	VE6_HPV68	Protein E6	E6	HPV68	158AA
P06927	VE5_HPV16	Probable protein E5	E5	HPV16	83AA

Table 1: selected sequences for high -risk HPV

CLUSTAL W

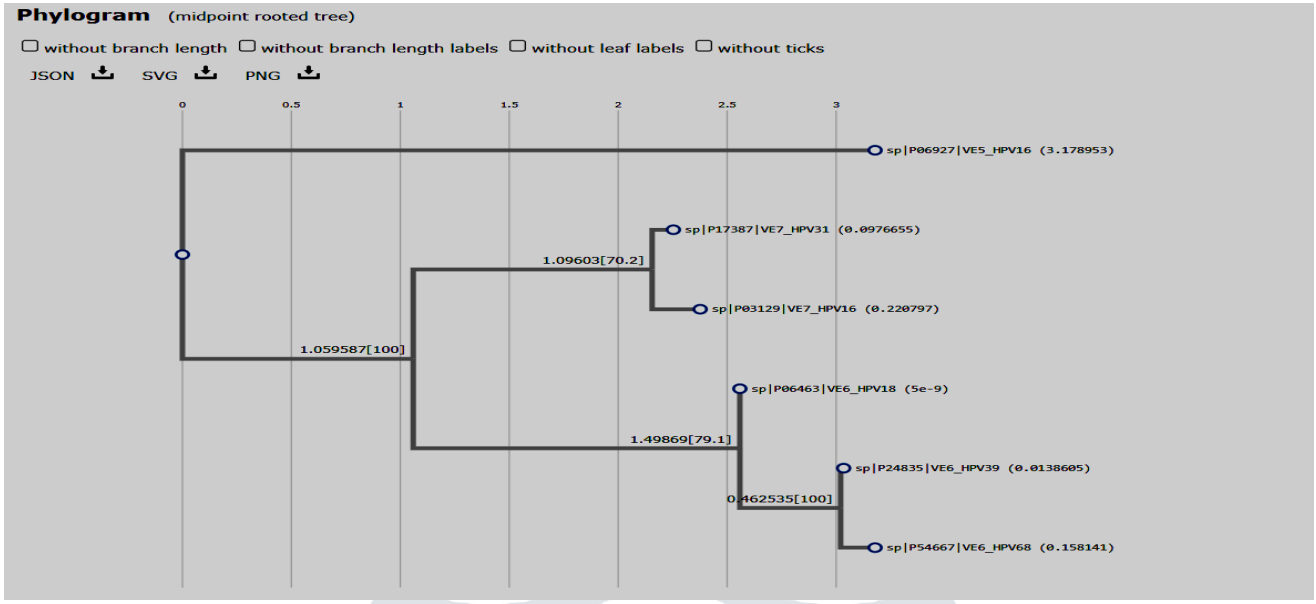

Fig.1: Phylogram from Clustal W

Clustal Omega

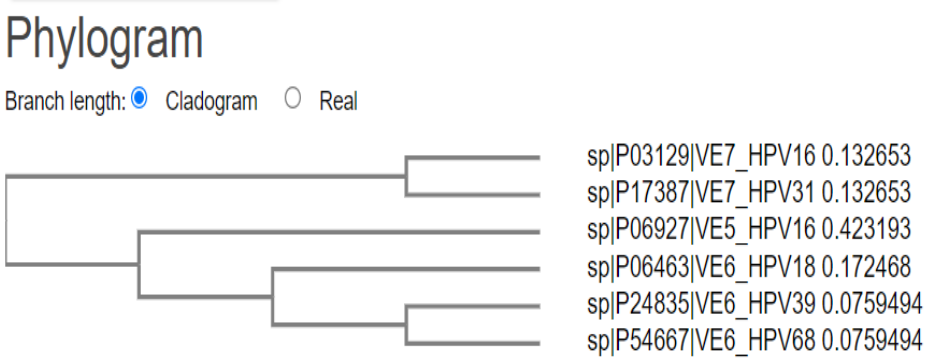

Fig.2: Phylogram from clustal omega

The results showed that the most distantly evolved gene is the HPV E5 gene. Based on the similarities of the E6 gene it is seen that HPV 39 and HPV 68 are closely evolved and they have evolved from HPV 18 E6 gene. However, this study shows a separate evolution of the E7 gene.HPV 16 and HPV 31 E7 genes are closely evolved. All these types of alpha HPV viruses are known to cause cervical cancer. From the above phylogram, it can also be inferred that the E5 gene of HPV 16 and E6 of HPV 18 have evolved from a common point.

The 3 most prevalent types of HPV in cervical cancer are HPV 16,18 and 45 which account for 75% of squamous cell carcinoma and 94% of adenocarcinoma. HPV 45 is a member of the HPV 18 species. HPV 45 accounts for 5% of cervical cancers (8). Hence in this study HPV 16 and HPV 45 were analyzed separately to see their evolution. A common target identification purpose and to understand the evolutionary pattern of the most prevalent HPV s and the most carcinogenic.

GEN BANK	ACCESSION	PROTEIN	GENE	ORGANISM	LENGTH

		NAME	NAME		
CAD1814427.1	CAD1814427	E1	HPV45	HPV	643AA
CAD1814428.1	CAD1814428	E2	HPV45	HPV	368AA
CAD1807061.1	CAD1807061	E4	HPV45	HPV	90AA
CAD1807062.1	CAD1807062	E5	HPV45	HPV	73AA
CAD1814425.1	CAD1814425	E6	HPV45	HPV	158AA
CAD1814426.1	CAD1814426	E7	HPV45	HPV	106AA
CAD1814430.1	CAD1814430	L1	HPV45	HPV	536AA
CAD1814429.1	CAD1814429	L2	HPV45	HPV	463AA
AYV61476.1	AYV61475	E1	HPV16	HPV	649AA
AYV61477.1	AYV61477	E2	HPV16	HPV	365AA
AYV61479.1	AYV61479	E5	HPV16	HPV	83AA
QCF46739.1	QCF46739	E6	HPV16	HPV	158AA
ABK32512.1	ABK32512	E7	HPV16	HPV	98AA
AYV61481.1	AYV61481	L1	HPV16	HPV	531AA
AYV61480.1	AYV61480.1	L2	HPV16	HPV	473AA

Table 2 : secquences of HPV 45 and HPV 16

Clustal W

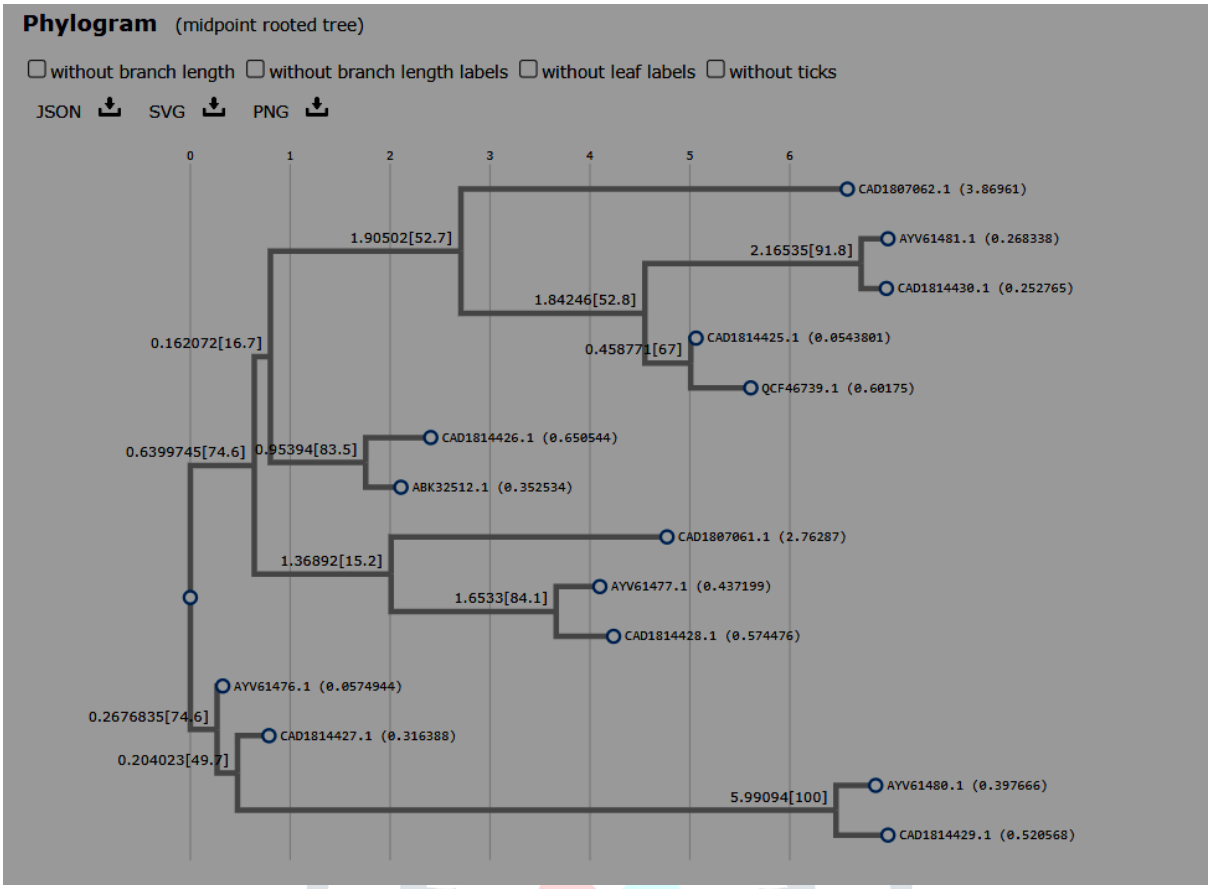

Fig. 3: HPV 45 and HPV 16 phylogram from Clustal W

Clustal Omega

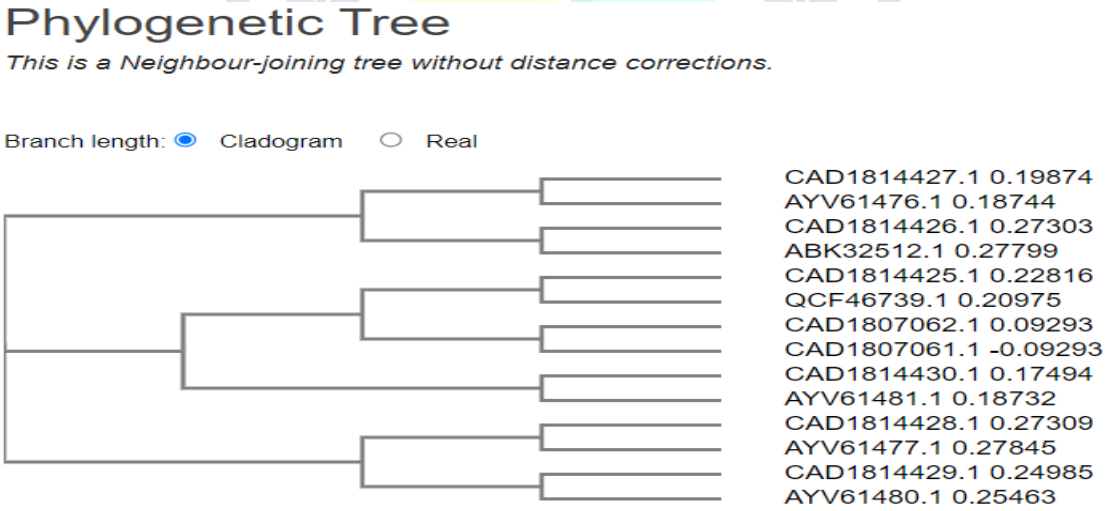

Fig. 4: HPV 45 and HPV 16 phylogram from Clustal Omega

The results from the above phylogram show that all the genes except the E5 gene of HPV 45 and the E1 genes of HPV 16 and HPv45 evolved closely and originate from a common point. Making E5 a target for any distinct primer design for HPV 45. It is also seen that the HPV E4 gene is closely evolved with the E2 genes of HPV 16 and HPV 45.

Blast results

S.NO	DESCRIPTION	MAX SCORE	E-VALUE	IDENTITY	POSITIVES
1	E6 Protein (HPV 16)	326	2e-112	100%	100%
2	E6(Alpha papilloma virus)	325	3e-112	99.37%	99.37%
3	E6 (HPV 31)	325	3e-112	99.37%	99.37%
4	E6(HPV18)	325	4e-112	99.37%	99.37%
5	E6(HPV 45)	325	4e-112	99.37%	99.37%

Table 3: HPV E6 high -risk blast results.

The blast of the HPV E6 the main onco protein showed that most of the E6 proteins of the main oncogenic viruses are similar. So can be chosen as a common target. Also, it can be inferred that the mechanism of all the E6 proteins in the infection is the same. i.e mechanisms of E6 proteins in the infection process are closely related.

Low-risk HPV

HPV low risk also causes genital warts and are persistent in infection hence it becomes a necessity to analyze the evolution of low-risk HPV as well.

GEN BANK ID	ACCESSION	PROTEIN NAME	GENE NAME	ORGANISM	LENGTH
ALT54574.1	ALT54574	HPV6	E5	HPV	72AA
ALT54551.1	ALT54551	HPV6	E6	HPV	159AA
ALT54570.1	ALT54570	HPV6	E7	HPV	98AA
QEEE83892.1	QEEE83892	HPV11	E5	HPV	91AA
QEEE83893.1	QEEE83893	HPV42	E5	HPV	74AA
UXE30374.1	UXE30374	HPV11	E6	HPV	150AA
UXE30375.1	UXE30375	HPV11	E7	HPV	98AA
CAD1814609.1	CAD1814609	HPV42	E7	HPV	93AA
CAD1814550.1	CAD1814550	HPV42	E6	HPV	150AA
CAD1814275.1	CAD1814275	HPV43	E6	HPV	155AA
CAD1807853.1	CAD1807853	HPV43	E7	HPV	99AA

Table 4 : E6 and E7 of HPV Low-Risk

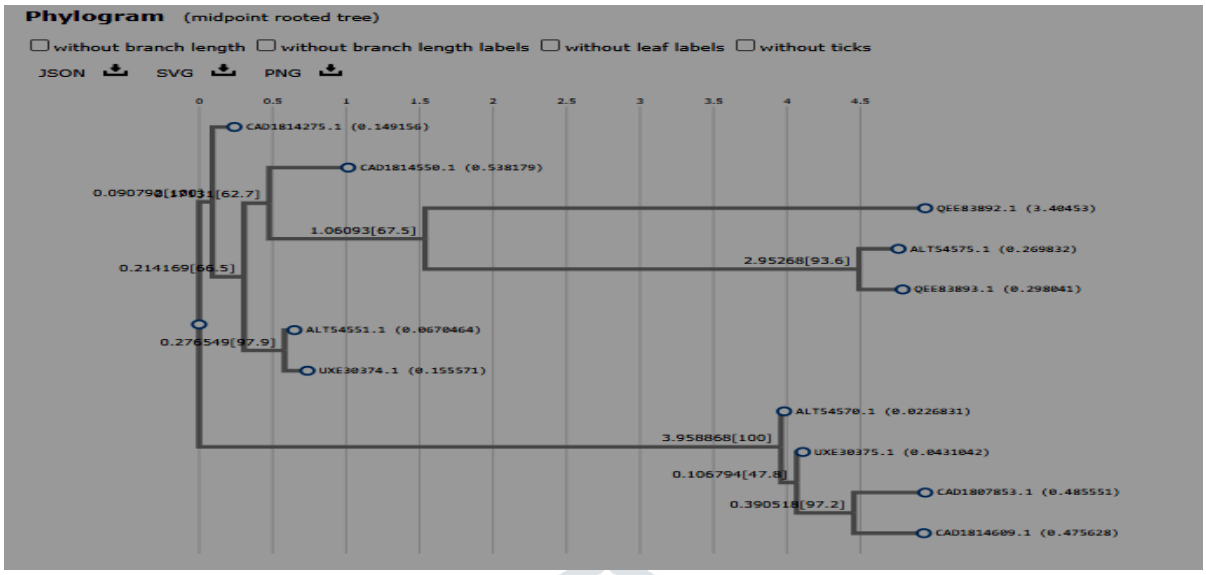

Fig.5 :Phylogram of low risk from clustal w

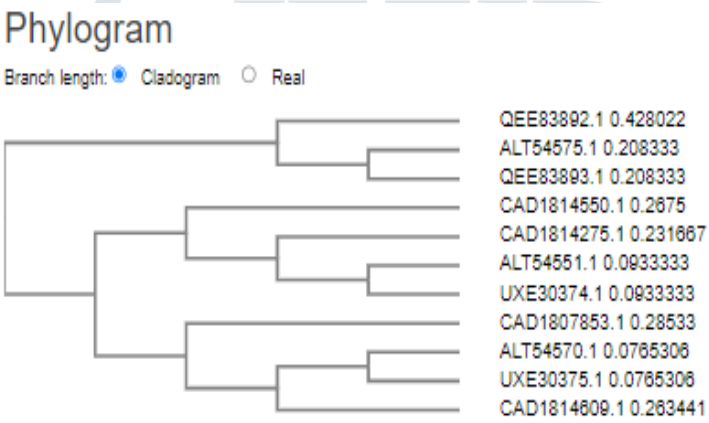

Fig.6 : Phylogram of low risk from clustal omega

The results of the study show that in the low-risk groups as well the E6, E7, and E5 genes of the most common types of low-risk HPV evolved similarly. The E5 gene is the most distantly evolved and E6 and E7 genes evolve from a common ancestor.HPV 42, HPV43, HPV 6, and HPV 42 are taken for the study.

Discussion and Conclusion

Based on the characteristics and properties, a genus is classified. For HPV so far 5 genera have been classified namely alpha, Beta, Gamma, nu, and mu. While Delta-papillomavirus, Epsilon-papillomavirus, Zeta-papillomavirus, Eta-papillomavirus, Theta-papillomavirus, Iotapapillomavirus, Kappa-papillomavirus, Lambda-papillomavirus, Xi-papillomavirus Omikron-papillomavirus, and Pipapillomavirus are responsible for diseases in animals (23) .The members of the alpha papillomaviruses are the causative agents of cervical cancer. Recent studies show that the evolution of these cancer-causing viruses occurred half a million years ago from the most recent common ancestors. The initial stage of evolution is the function adaptation of the virus to the host ecosystems and the co-evolution process (23). E7 protein and E6 protein have a similar structure and hence it is noted that they share an evolutionary history (4). Classification of the virus has been carried out with the distinction based on the L1 protein-encoding gene (9). The side chains around the axial cavity are almost completely conserved. Likewise the polypeptide chains of L2.HPV viral genes are commonly evolved and can be

chosen as a target for identifying HPV infection commonly. HPV E5 and L1 genes may be chosen as a target for distinct identification.

Summary

The E6 gene is conserved in HPV high -risk . the evolution takes part in the L1 gene. The evolution pattern of the genes are related to each other. E6 can be chosen as a molecular target common for HPV high -risk and also as a drug target .L1 gene can be chosen for targeting the different sub types.

Conflict of Interest

The authors declare no conflict of interest in the above study.

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