

Systematic Review

Molecular epidemiology of human papillomavirus variants in cervical cancer in India

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Received January 31, 2024; Accepted July 29, 2024; Ahead of print January 16, 2025; Published ***** **, 2025

Background & objectives: Cervical cancer (CC) has been documented as the fourth most common cancer worldwide. Persistent infections with high-risk human papillomavirus (hr-HPV) have been suggested in the development of CC. Although prophylactic vaccines are available for the prevention of prevalent hr-HPV types, intra-type variations exist within a particular HPV type that has varying oncogenic potential as well as the mechanism of pathogenicity and varying neutralization by antibodies. Therefore, we carried out a systematic review to determine the distribution of HPV intra-typic variations in different geographical locations of India and their reported implications.

Methods: Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines were followed to retrieve relevant articles from the standard databases using appropriate keywords. Consequently, 17 articles were included in the current review after screening based on inclusion and exclusion criteria.

Results: The majority of articles included in this review reported variations within the HPV16 E6 gene, followed by the L1 and E7 genes. Analysis of available data indicated the differential regional distribution of some variations. These variations have also been reported to impact the biological functions of various viral proteins.

Interpretation & conclusions: The distribution of lineages varied with the different genomic regions sequenced. Additionally, there were certain unique and common variations in the HPV genome with respect to geographical regions. Hence, we suggest the identification of region-specific variations for the development of diagnostic and prognostic interventions.

Key words Asian American variants - European variants - HPV - intratypic variants - lineage - sublineage - variations - 350G variant

Cervical cancer (CC) is the fourth most common cancer and the fourth leading cause of cancer deaths among women worldwide¹. The incidence of CC is disproportionately high among low- and middle-income countries. In India, it is the third most frequent

cause of cancer mortality². Persistent infections with high-risk human papillomavirus (hr-HPV) have been documented as a major cause of CC and its involvement in other types of cancers³. HPV is a circular, non-enveloped, double-stranded DNA virus infecting basal

Table I. Properties and biological functions of various HPV genes⁵⁻⁷

Viral genomic regions	Properties	Biological functions
L1	The L1 proteins are mainly expressed during the later stages of viral infections. It consists of variable & constant regions, where the variable region carries surface-specific antigenic epitopes that cause the production of neutral antibodies	The major capsid protein (L1) forms the pentameric monomers that comprise 5L1 and 1L2 protein, which ultimately forms 72 capsomeres that cover the virus. It can produce HPV-specific antibodies due to the presence of multiple surface epitopes
L2	Minor capsid protein along with L1 forms the capsid of the virus	It is responsible for interaction with host receptors & facilitates endocytosis of the viral genome
E1	It is the highly conserved protein among HPV types and is expressed during early infection	It is responsible for viral replication. It also regulates epigenetic modulations within the cell as well as is also reported in the immune response
E2	It consists of two domains, the N terminus conserved domain, involved in transactivation and DNA replication, and the C terminus DNA binding domain that facilitates dimerization.	The E2 protein along with the E1 protein is involved in viral DNA replication and transcription. It is also involved in the repression of E6 and E7 gene expression by binding to its promoter within the LCR
E4	The E4 gene is present within the E2 gene and is translated as E1^E4 fusion protein. It consists of a leucine cluster motif in its N terminus that aids in association with keratin.	The E4 protein is involved in viral release and transmission
E5	It is a membrane-bound protein that consists of 3 domains	It is involved in hyperproliferation and cancer progression. It also induces angiogenesis, suppresses tumour suppressor proteins, and promotes anti-apoptosis
E6	E6 protein consists of 2 zinc finger domains, flanked by 4cys-X-X-cys motifs, as well as a PDZ-binding motif	It is mainly involved in p53 degradation, to evade cell death, proliferation, cell invasion, immune evasion, and immortalization
E7	E7 protein consists of three conserved regions. The conserved region near the C-terminus encodes the zinc finger domain flanked by 2CXXC motifs	The E7 protein is involved in inactivation of pRB and the downregulation of E2F. it also causes cell cycle progression, cell proliferation, cell invasion, and inflammation
LCR	LCR can be subdivided into 3 segments namely the 5' segment, central segment, and 3' segment, which are sites for binding to viral and host factors	LCR is responsible for viral replication, regulation of viral transcription, and binding to various viral and host factors

LCR, long control region; HPV, human papillomavirus

keratinocytes of the host⁴. HPV genome can be divided into three regions, namely, (i) early gene region (E) consisting of E1, E2, E4, E5, E6, and E7, (ii) late gene region (L) including L1 and L2 genes, and (iii) non-coding, long control region (LCR). In addition, a smaller non-coding region between E5 and L2 genes is referred to as non-coding region 2 (NCR2). The important functions of these regions are given in table I⁵⁻⁷. These variations have also been suggested to modulate their oncogenic potential and may also facilitate immune evasion^{8,9}. HPV can be classified into various types depending upon at least 10 per cent difference in its highly conserved L1 gene¹⁰. Furthermore, nucleotide variations within HPV types can be used to classify each type into variant lineages and sub-lineages^{11,12}. Lately, the HPV 16 complete genome has been

classified into 4 lineages, named A, B, C, and D, which are further divided into sub-lineages, A1-3 (European), A4 (Asian), B1-4 (African1), C1-4 (African2), D1 (North American), D2-3 (Asian American) and D4¹³. Different geographical prevalences of certain intra-type variants have been reported⁸. Consequently, intra-type variants differ in their oncogenic potential and mechanism of oncogenicity¹⁴, as Asian American variants of HPV16 have been reported to retain the E2 gene, suggesting an alternative mechanism to regulate E6 and E7 transcription¹⁵. Studies have also reported reduced sensitivities of HPV variants to cross-neutralization by vaccine antibodies and/or mAb raised against A antigens¹⁶⁻²⁰.

Therefore, the identification of HPV variants may be vital for the development of new diagnostic and

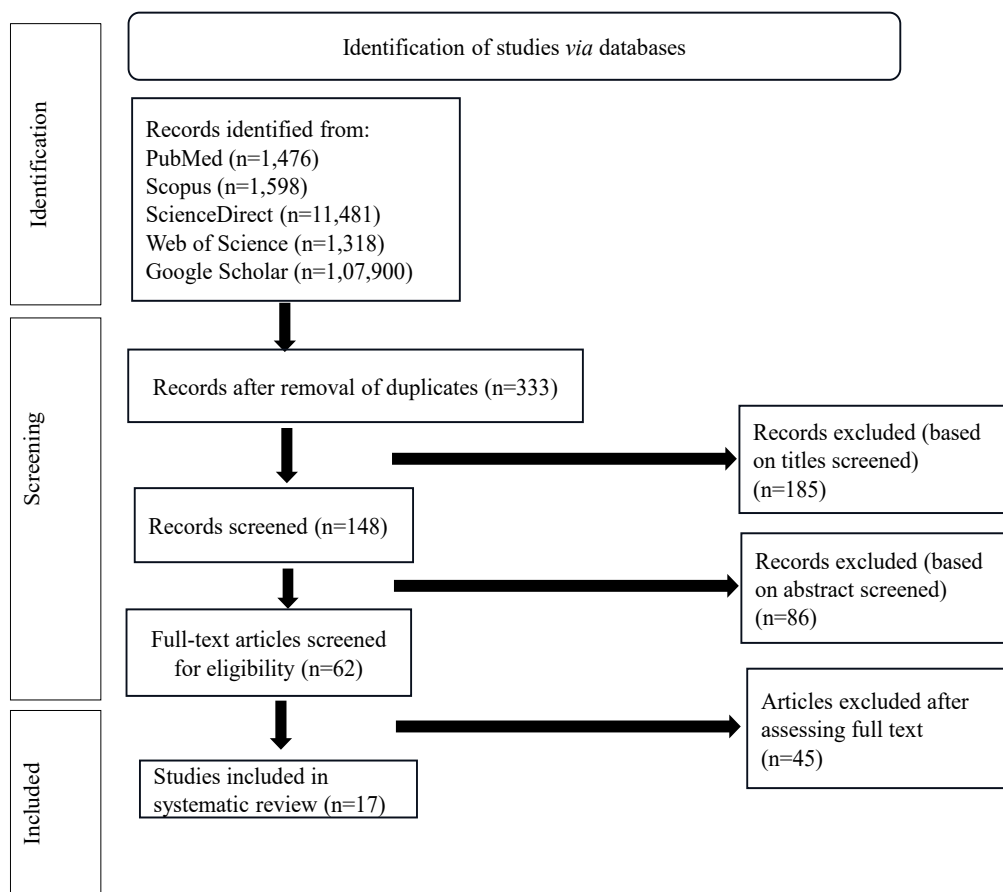


Fig. 1. PRISMA flow diagram for the study selection process.

therapeutic interventions. Hence, we carried out the current review to determine the distribution of HPV variants within the Indian population and their reported implications, which can subsequently be instrumental in designing population-specific interventions.

Materials & Methods

The study followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. Articles were retrieved from PubMed, Scopus, Science Direct, and Web of Science on September 9, 2023, and again on November 4, 2023. Key words used for assessing relevant literature included ‘HPV variants in cervical cancer’, ‘nucleotide variations AND HPV AND cervical cancer’, ‘single nucleotide polymorphisms AND HPV AND cervical cancer’, and ‘Human papillomavirus lineages AND cervical cancer’. Grey literature sources like Google Scholar were also consulted to ensure that all pertinent articles were included. A total of 333 articles were

obtained after removing duplicates through titles. The remaining articles (n=148) were then screened through their abstracts, and a total of 62 articles were obtained, the full text of which were studied further to exclude irrelevant articles. Finally, 17 relevant articles pertaining to the objective of the current systematic review were included (Fig. 1).

Inclusion criteria: The inclusion criteria comprised research articles involving participants diagnosed with abnormal cytology or with CC, were exclusively from India, availability of full text, articles in the English language, and those that were relevant as per the objective of the current review.

Exclusion criteria: Exclusion criteria were articles in which participants were coinfecting with other pathogens, studied in countries other than India, different diseases, or in women with normal cytology, and articles in which the country of origin of samples could not be determined through text.

Quality Assessment: The methodological evaluation of the given studies was based on the Quality assessment of observational studies^{21,22}. Depending on the study design of the included articles, a total of 10 items were selected for cohort studies, while 12 items were selected for case-control studies. The observational cohort studies with a score of >7 were regarded as high-quality studies, with a score of 4 to 7 as medium quality and below 4 as low-quality studies. Similarly, the observational case-control studies with scores >8 were regarded as high-quality studies, with scores 4 to 8 as medium and below 4 as low-quality studies. The evaluation of the studies was done through discussion, and the results of methodological quality assessment are provided in supplementary tables I and II

Distribution of studies into different geographical regions: According to geographical location, the relevant studies were segregated into eastern, western, northern, southern, and central regions, and studies involving participants from multiple locations in India. Details of the participants recruited, and the HPV genome region studied have been compiled in table II.

Results

Quality assessment of the studies: The quality assessment of the included studies found that seven of ten cohort studies were of high quality, and three were of medium quality. Similarly, all the case-control studies were of high quality.

Distribution of HPV variants among various geographical regions: Out of the total studies, most reported HPV16 variants, while a single study reported HPV18 variants. The gene studied the most was the E6 gene, followed by L1 and E7, which had five studies each. However, no study to date reports the epidemiology of variants of other prevalent HPV types in the Indian population. Distribution of nucleotide variations as well as lineages of HPV16, reported in the included studies, have been compiled in table III and IV, respectively. Furthermore, the nucleotide variations and lineage distribution of HPV18 have been compiled in table V.

HPV 16 variants:

Variants in East India: A study reported 20 variations within the E2 gene with C3007T, G3058A, C3236G, A3253G, and A3605G as prevalent among controls,

depicting that these may confer reduced pathogenicity in HPV16 variants²³. Certain novel variations were also observed. Additionally, specific E2 variations were used by the authors to determine lineages, which suggested that the majority of the variants belonged to the European (A1-3) lineage followed by the Asian American lineage (D2-3). It was also reported that E2 disruption was more frequent among cases. In addition, 22 variations in the long control region (LCR) region were also determined. Variation T7450C was reported to be present in E2 binding site-IV and was prevalent among cases. In addition, most variants having this variation had no other variation within the LCR or E2 gene, thus indicating inter-gene co-variations within individual isolates. The majority of LCR variants were reported to belong to European (A1-3) lineage, followed by Asian American (D2-3).

Another study from eastern India observed that 262 single nucleotide variants were present within the studied samples, among which 20 non-synonymous variations were deleterious²⁴. The most prevalent variations are depicted in table III (with MAF ≥ 0.05 ; the variations whose synonymous/non-synonymous status could not be deduced were omitted from the table). The lineages and sub-lineages were assigned as per E2 (T3694A), E6 (A532G) and LCR (T7743G, G7834T) variants. The most prevalent lineage among the samples was European (A1), followed by North American (D1) and Asian American (D2). The D1 and D2 sub-lineages were reported to be exclusively present within the CC cases. Furthermore, it was reported that lineages A and D could be distinguished by 35 bi-allelic variants, while 6 variants could distinguish D1 and D2 sub-lineages. Subsequently, it was observed that certain variants from the A1 sub-lineage had differential distribution among malignant and non-malignant samples and could be associated with pathogenicity. To further understand the viral genomes in the Indian population, network analysis was performed, and it was found that four haplotypes were present among the A1 sub-lineage, among which two haplotypes were specific to cases. Additionally, certain nucleotide variations were co-existing. The propensity of D lineage towards integration of the HPV genome was suggested. Consequently, the presence of E5 variants (3979C, A4042G) and LCR region variations (7577T) in conjunction with E6 350G variations as risk alleles suggested the polygenic aspect of viral contribution to disease.

Table II. Details of the studies included in the review

Regions	Study	Yr	Sample size	HPV	Gene	
East India	West Bengal	To determine the variations within the E2 and LCR regions	2006 ²³	49 ISCC cases and 23 controls	16	E2
				42 ISCC cases and 19 controls	16	LCR
	West Bengal	To determine distinctive features of HPV16 lineages <i>wrt</i> nucleotide variations, viral load and E7 gene expression	2022 ²⁴	145 ISCC and 24 controls	16	Whole genome
West India	Mumbai	To determine the prevalence of molecular variants and their impact on disease outcome	2014 ²⁵	62 cervical cancer patients	16	E6
North India	New Delhi, Aligarh	To analyze variations within the viral genome along with its association with age, histopathological grade, and oncogenic potential	2008 ²⁷	31 cervical cancer patients	16	L1
				60 cervical cancer patients	16	E6
				60 cervical cancer patients	16	E7
				60 cervical cancer patients	16	LCR
	North India	To determine the predominance of HPV genotypes, variant classes of hr-HPVs, and viral load among various sample groups	2009 ²⁸	74 ICC 9 SIL 9 cytologically normal controls	16/18	E6/E7
	Delhi	To determine gene variants and predict epitopes for common MHC I and MHC II alleles present	2015 ³⁰	221 cervical cancer cases	16	E6
South India	Thiruvananthapuram	To examine the association of variations in HPV oncogenes with disease status	2002 ³¹	111 patients with HSIL and ICC 32 controls with benign or LSIL	16	E6/E7
	Vellore	To determine the presence of specific variations within the HPV gene, that have been reported to be significant for biological and immunological functions	2004 ³²	76 ICC patients and 16 CIN	16	E2
	Karnataka	To determine the prevalence of HPV types, and of different variants of HPV from rural women	2015 ³³	15 normal 24 SIL 51 malignant	16	L1
Central India	Chattisgarh	To analyze variations in the HPV oncogenes as well as their effect on protein structure	2021 ³⁴	21 cervical cancer patients	16	E6, E7

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Regions	Study	Yr	Sample size	HPV	Gene	
Multiregional	South and East India	To determine the frequency of the particular HPV variant among women	2005 ³⁵	50 CIN and ICC patients 20 controls	16	E6
	West Bengal, Chattisgarh, Manipur and Sikkim	To identify sequence variations within the HPV genome and their effects on oncogenicity, disease progression, and pathogenicity	2008 ³⁶	53 ISCC patients and 21 controls	16	Whole genome except E1
	Delhi, Kolkata, Bangalore, Mumbai, Thiruvananthapuram, Vellore	To determine gene variations among cervical neoplasia patients	2009 ³⁷	412 SCC	16	E6, E7 and L1
	West Bengal, Chattisgarh, Manipur and Sikkim	To extend the above findings by Bhattacharjee ²⁶ , variations within the entire HPV16 genome were assessed in the episomal HPV genome. In addition, the association of synonymous variations with pathogenesis as well as genes comprising such variations were identified and the significance of variations within non-coding regions was also evaluated.	2013 ³⁹	70 ISCC cases with intact E2 29 controls	16	E1
	Pune, Belgaum, Chennai	To characterize HPV gene variants and lineage/sub-lineage distribution and its correlation with sequence, structure, and functional implications.	2020 ⁴⁰	133 women undergoing screening Normal-43 LSIL-45 HSIL-45	16	L1
	Pune, Belgaum, Chennai	To determine variations within the HPV genome and its associations with different grades of neoplasia as well as the impact of these variations on the regulation of viral transcription.	2022 ⁴¹	54 normal 50 LSIL 59 HSIL (as per assigned lineage)	16	LCR
	Retrieved samples from Pune, Belgaum and Chennai	To identify variations within the HPV gene and its association with disease. Additionally, B- and T-cell epitopes in L2 were identified and the impact of found variations within epitopes was also determined.	2022 ⁴²	41 normal, 45 LSIL, and 62 HSIL	16	L2

ISCC, invasive squamous cell carcinoma; ICC, invasive cervical cancer; SCC, squamous cell carcinoma; SIL, squamous intraepithelial lesion; LSIL, low grade SIL; HSIL, high grade SIL; CIN, cervical intraepithelial neoplasia

Table III. Distribution of HPV 16 nucleotide variations/amino acid substitutions within different regions of India

Genomic regions	Nucleotide variations/amino acid substitutions		References
	Synonymous variations	Non-synonymous variations (amino acid substitutions)	
East India			
L1	G5696A, C5756G, A5816T, T5855C, T5909C, A6023T, 6028delA, 6049insA, 6092delA, 6105delA, T6245C, A6314G, C6539A, *C6557T, A6559T, 6560delT, 6561delA, 6606insT, 6626insT, 6638delT, *A6665C, 6669delT, G6719A, A6779T, C6824T, *C6852T, 6857delA, C6863T, 6871delG, 6909delA, 6918delC, 6934delC, T6966C, C6968T, C6969T, T6972C, G6978A, A6983C, 6988delT, 6989delA, 6990delA, *G6992A, A6994G, T6999G, G7008A, 7011delC, C7017A, 7027delT, 7049delA, G7058A, G7062C, A7073T, 7075delT, 7076delT, 7077delA, 7098delA, C7106A, T7130G, 7135delG, G7144C	*C5562G (Q2E), A5620G (Y21C), T5629C (F24S), T5638A (M27K), A5797C (K80T), G5829A (V91I), *C5862T (H102Y), *C6163A (T202N), G6171A (A205T), A6178C (N207T), C6240G (H228D), A6432G (T292A), A6445C (N296T), C6564A (P336T), G6652C (S365T), *A6693C (T379P), A6801T (T415S), 6901insAC (448insS), 6950delGT (465delD), G7033C (R492P), *G7058T (L500F), G7090A (R511Q), A7132C (K525T)	37
L1	T5675C, *G5698A, A5755C, *T6247C, A6316G, T6319C C6370T, A6391G, A6425C, C6541A, *A6565G, *C6559T, *A6667C, *G6721A, C6826T, *C6854T, *C6865T, T6889G, A6952G, *C6970T, *G6994A, T7027G, G7032A, A7072T, T7078C, A7141C	*C5864T (H76Y), *C6165A (T176N), G6173A (A179T), *A6180C (N181T), C6182G (P182A), *A6434G (T266A), T6438G (V267G), A6445C (N296T), G6458A (D274N), G6582A (R315Q), T6689G (S351A), *A6695C (T353P), *A6803T(T389S), T7028G (L464V), A7148C (K504Q)	24
L2	A4242G, A4266C, *T4281C, A4299C, *A4410G, *G4428T, T4446G, *T4452C, *G4461A, A4467C, A4468C, C4525T, T4545G, *A4599C, A4626T, A4632C, *T4644A, T4647G, T4695C, *C4725T, A4788C, A4887G, G4938A, A4944G, *A4950G, *A5034T, A5073T, G5142A, T5286A, *G5379A, A5409G, T5412G, T5475A, T5478C, *C5487T, A5517G, T5523C, A5532G, A5622G	C4253T (S6F), *T4600C (S122P), G4621A (D129N), G4654A (D140N), A4693C (T153P), A4811C (N192T), A4820G (E195S), *A4969G (T245A), G5008A (E258K), A5027C (N264T), T5041C (S269P), C5042A (S269Y), A5048C (N271H), T5060A (I275N), A5063C (N276T), A5152C (I306L), A5250C (E338D), A5256T (E340D), A5356C (I374L), T5357G (I374S), C5369T (S378V), *T5386G (S384A), *G5389A (V385I), A5466C (L410F), A5492C (I418T), *T5495C (I420T), A5505C (Q423H), *G5506A (A424T), A5518C (I428L), *T5368G (S378V), *C5564G (A443G), G5609A (R458Q)	24
L2 UTR/ NCR2	A4150C, *C4151A, *A4157G, *T4158A, *C4164T, A4184T, T4186A, T4222C, T4231G, A4233C, A4234C		24
E1	*T921C, T1221C, T1446C, G1941A, T2301C, T2343C, *C2344T, T2470C, T2478C, T2778C	C874T (P4S), *G1293T (Q143H), *T1297A (L145I), *G1363A (G167S), *T1421C (I186T), *A1842G (I326M), G2337A (M491I), *G2650A (E596K)	24
E2	*C3007T, *G3058A, A3187C, *A3253G, A3538C, T3694A	<u>C2988T (T78M), A3025C (E90D), C3161T (H136Y), *C3236G (H161D), *A3605G (Q286D), T3517A/C (T254K/N), T3566G (F271V), C3684A (T310K), A3181C (E142D), G3182A (A143T), T3224A (L157I), G3249A (R165Q), C3516A (T254K)</u>	23
E2	T2914C, C3007T, G3058A, T3172G, *A3181C, A3187C, A3253G, T3259C, T3313G, T3664C, *C3684A, *T3694A, *T3706C, *C3787A, C3800G, *T3805G, T3820C, *T3847C	C2988T (T78M), C3161T (H136Y), *C3159A(T135K), T3172G (I139M), *G3182A (A143T), A3208C (Q151H), *T3224A (L157I), C3236G (H161D), *G3249A (R165Q), A3285C (K177T), G3778T/C (W341C/W341C)	24

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Genomic regions	Nucleotide variations/amino acid substitutions		References
	Synonymous variations	Non-synonymous variations (amino acid substitutions)	
E2/E4	A3362G, *A3366C, T3371C, C3377G, T3387C, *C3410T, G3413A, A3605G	*A3362G (N203D/A7A), A3366C (E204A/K9Q), T3371C (S206P), *C3377G (P208A/L12L), T3387C (I211T), *C3410T (P219S), G3413A (A220T), *C3516A (T254N/L59T), *T3517C (T254N/L59T), *A3538C (S261S/Q66P), *T3566G (F271V/H75Q), C3576T (S274L/H79Y), G3585C (G277A/D82H), A3605G (N284D)	24
E6	*T286A, *A289G, *A532G	*G145T (Q14D), *C335T (H78Y), *T350G (L83V), G176A (D25N), G188C (E29Q)	24
E6	A181C, T286A, A289G, G522DEL, A532G, C537DEL	G145T (Q14H), G176A (D25N), G188C (E29Q), <u>T311A (Y70N)</u> , *C335T (H78Y), <u>A336G (H78C)</u> , *T350G (L83V), <u>C506G (R135G)</u>	37
E7	G666A, T687C, *T789C, *T795G	*T732C (F57V), C790T (R76C)	24
E7	G666A, T687C, T730G, *T789C, *T795G	*T732C (F57V), G823A (G88R), <u>T844DEL (Frameshift)</u> , <u>G849C (Q98H)</u> , A852DEL (Frameshift, K97N, P98H)	37
LCR	*T7450C, C7394T, C7395T, A7482G, A7485C, G7489A, Int-del T 7497, C7689A, T7743G, A7729C, C7764T, C7786T, G7868A, G7521A, A7550G, T7568G, C7669T, G7703T, T7713G, T7714G, T7775A, G7826A		23
LCR	A7168G, T7176G, T7187C, *G7193T, A7227C, A7233C, A7376T, C7394T, C7395T, T7401C, G7427A, G7435A, *T7450C, A7482G, A7483C, *A7485C, *G7489A, *G7521A, A7550G, C7577T, C7655A, *C7669T, *C7689A, T7713G *A7729C, *T7743G, *C7764T, A7773C, T7775A, *C7786T, C7792T, G7826A, T7827A, G7834T, G7868A, C7886G, C13T, C24T, A93T, A98C		24
West India			
L1	T5673C, G5696A, T5855C, T5909C, 6020delC, A6023T, 6105delA, T6245C, A6314G, C6265T, A6389G, C6539A, 6554delA, *C6557T, *A6665C, 6706delA, G6719A, *C6852T, T6860C, C6863T, A6935T, A6947G, C6968T, A6983C, *G6992A, T7025G, G7058A, T7076C, A7154G	*C5562G (Q2E), C5600A (Y14X), C5609A (D17E), *C5862T (H102Y), A6021G (T155A), G6024A (E156K), *C6163A (T202N), G6171A (A205T), A6178C (N207T), C6240G (H228D), G6261T (D235Y), C6388T (R277L), A6432G (T292A), G6495T (A313S), 6563delA (K335X), *A6693C (T379P), A6801T (T415S), G6879A (E441K), 6901insAC (448insS), G6945A (E463K), 6950delGT (465delD), G7008C (D484H), *G7058T (L500F), A7087C (K510T), G7090A (R511Q), T7150A (L531Q), A7154C (X532Y)	37
E6	<u>A187G</u>	*T350G (L83V), *350T, <u>T310G (F69L)</u> , <u>A512T (M137L)</u>	25
E6	A111G, T286A, A289G, A320DEL, INS342G, G522T, A529G, T530A, A532DEL, A532C, A532G, A562DEL	A131G (R10G), G145T (Q14H), C158A (L19M), G176A (D25N), A182T (I27L), C315G (S71C), *C335T (H78Y), *T350G (L83V), C479T (H126Y), T521C (C140R), T521G (C140V), INS524A (R141K), C531T (S143I)	37
E7	*T789C, *T795G	A619T (T20S), *T732C (F57V), A746DEL (Frame shift), <u>G829DEL (V90C, C91A)</u> , <u>T844DEL (Frame shift)</u>	37
North India			
L1	<u>A6667C</u> , <u>A6691G</u> , A6721G, C6854T, T6889G, C6970T, G6994A, C6865T	*A6695C (T353P), A6803T (T389S), A6964C, <u>C6906T (S423F)</u> , <u>A6924C (Q429P)</u>	27
L1	T5673C, T5681C, G5696A, A5795G, A5813G, A5816T, A5834G, T5909C, C5967T, A6023T, A6068G, A6182C, T6245C, G6278A, C6365T, A6389G, A6452G, A6518G, C6539A, *C6557T, *A6665C, G6719A, A6779T, *C6852T, C6863T, T6887G, G6936A, C6968T, *G6992A, T7076C, A7094G	* <u>C5562G (Q2E)</u> , A5620G (Y21C), *C5862T (H102Y), G5871T (D105Y), *C6163A (T202N), A6178C (N207T), C6240G (H228D), C6388T (R277L), A6432G (T292A), G6495T (A313S), T6573A (L338I), T6634A (V359D), *A6693C (T379P), A6801T (T415S), G6879A (E441K), 6901insAC (448insS), 6950delGT (465delD), G7008C (D484H), *G7058T (L500F), G7090A (R511Q)	37

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Genomic regions	Nucleotide variations/amino acid substitutions		References
	Synonymous variations	Non-synonymous variations (amino acid substitutions)	
E6	T286A, A289G, A532G	G145T (Q14D), C335T (H78Y), *T350G (L83V), <u>T527A (S142T)</u>	27
E6	T286A, A289G, A532G	T109C, A131G (R10G), G145T (Q14D), <u>A169C (T-P)</u> , G176A (D25N), T178G (D25E), <u>G293A (D-N)</u> , C335T (H78Y), T350G (L83V), 403, <u>G507A (R-E)</u> , <u>G525T (R-I)</u>	28
E6	T286A, A289G	G145T (Q14H), *C158T, C335T (H78Y), *T350G (L83V)	30
E6	A169C, G267A, T286A, A289G, G293A, G507A, G525T, A532G	A131G (R10G), *G145T (Q14H), G176A (D25N), T178G (D25E), C315G (S71C), *C335T (H78Y), *T350G (L83V)	37
E7	G666A, T789C, T795G, T828C	T732C (F57V)	27
E7	<u>G663A</u> , <u>G826T</u> , A746T, T789C, T795G	A647G (N29S), T732C (F57V)	28
E7	G663A, A746T, *T789C, *T795G, T843C	A647G (N29S), *T732C (F57V), G823A (G88R), A826T (I89F), <u>T846C (S95L)</u>	37
LCR	*G7521A, <u>A7636C</u> , C7689A, *T7714G, C7792T, G7826A, A7839C, T7743G, *C7764T, <u>C13T</u> , C7669T, <u>C7678T</u> , A7729C, C7786T, <u>G7799A</u> , G7834T, C7886G		27
South India			
L1	DELETION AND INSERTION AT 6695, 6722,6733, 6736, 6737, 6738 and 6744	SNPs at 6722, 6742, 6743, 6759, *6760, *6726, *6729, *6730, *6732, *6738, *6739, *6741, *6744, *6759.	33
L1	55595insC, <u>T5597A</u> , G5636A, G5696A, T5801G, A5847C, T5906C, T5909C, T5999G, A6023T, 6028delA, A6068G, A6177C, A6200G, T6245C, 6253delG, 6283insA, G6302A, A6314G, C6539A, T6553C, *C6557T, A6561T, 6564delC, A6581C, A6581T, T6608C, 6658delA, *A6665C, G6719A, G6737T, *C6852T, C6863T, T6911A, T6914C, A6947G, C6968T, *G6992A, A7028G, G7058A, T7076C	*C5562G (Q2E), <u>A5568C (T4P)</u> , C5600A (Y14X), <u>A5602G (E15G)</u> , A5730C (N58H), *C5862T (H102Y), *C6163A (T202N), A6166T (N2031), G6171A (A205T), A6178C (N207T), C6240G (H228D), A6293C (E245D), C6352T (S265L), A6432G (T292A), A6445C (N296T), A6490C (N311T), C6502T (S315L), A6504C (N316H), T6560A (N334K), 6563delA (K335X), C6564A (P336T), C6565T (P336L), *A6693C (T379P), A6801T (T415S), 6901insAC (448insS), 6950delGT (465delD), A6961C (K468T), C6970A (T471N), A6997G (K480R), *G7058T (L500F), G7084A (G509E), 7093C (K512T)	37
L1	5605delA, 5640delT, G5696A, A5834G, T5909C, T6230C, T6245C, A6314G, A6452G, *C6557T, A6561T, 6654delA, 6663insA, *A6665C, G6719A, *C6852T, C6863T, A6891C, C6968T, T6971G, A6979G, *G6992A, G7058A, 7120insTC	*C5562G (Q2E), <u>T5597G (C13W)</u> , T5598G (Y14D), G5607A (D17N), *C5862T (H102Y), *C6163A (T202N), G6171A (A205T), A6178C (N207T), C6240G (H228D), A6432G (T292A), A6458G (D300E), T6560A (N334K), 6563delA (K335X), *A6693C (T379P), A6801T (T415S), 6901insAC (448insS), 6950delGT (465delD), G7008C (D484H), C7011A (L4851), *G7058T (L500F), T7110A (S518T)	37
L1	T5673C, A5687G, G5696A, A5702C, A5834G, T5909C, 6022delC, A6023T/C, G6059A, A6068G, 6087delT, T6245C, T6269C, A6314G, T6317A, A6389G, A6452G, T6482C, C6539A, A6554C, *C6557T, A6581T, A6656G, *A6665C, A6668G, G6719A, C6726T, G6836A, *C6852T, T6860C, C6863T, G6893A, A6938C, A6947G, C6968T, A6989G, *G6992A, G7058A, T7076C, T7130A, C7143A, T7145G	C5562G (Q2E), A5620G (Y21C), C5862T (H102Y), *C6163A (T202N), A6178C (N207T), C6240G (H228D), A6432G (T292A), 6563delA (K335X), *A6693C (T379P), A6801T (T415S), T6822C (S422P), 6901insAC (448insS), A6947C (E463D), 6950delGT (465delD), C6957T (L467F), *G7058T (L500F), C7117A (T520N), A7147C (K530T)	37
E2	A2983G, A3538C, T3664A, C3684A, T3694A, T3706C, G3778T	C3161T (H136Y), C3516A (T254N), T3517C (T254N), T3566G (F271V), T3371C(S206P), C3159A (T135K), A3362G (N203D), C3410T (P219S), G3449A, G3778A	32

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Genomic regions	Nucleotide variations/amino acid substitutions		References
	Synonymous variations	Non-synonymous variations (amino acid substitutions)	
E6		350T, *T350G (L83V), *C335T (H78Y), *G145T (Q14D), T419G (C106G)	31
E6	T286A, A289G, A508C, A532G, A536DEL	G145T (Q14H), T178G (D25E), T329C (Y76H), *C335T (H78Y), G491A (G130S), C528T (S142L)	37
E6	T254DEL, T265DEL, T279DEL, T286A, A289G, A532G	G145T (Q14H), T178G (D25E), <u>T179G (I26V)</u> , G188A (E29K), *C335T (H78Y), *T350G (L83V), A442C (E113D), A526T (R141S), T527A (S142T)	37
E6	T286A, A289G, A532G	G145T (Q14H), T183G (I27R), G205T (K34N), C245T (R48W), T308C (F69L), *C335T (H78Y), *T350G (L83V), T402C (L100S), G491A (G130S)	37
E7	T732C, T795G	*A647G (N29S), *C790T (R76C)	31
E7	*T789C, *T795G, T843C	A647G (N29S), *T732C (F57V), C747DEL (Frame shift), G842DEL (Frame shift), <u>T844DEL (Frame shift)</u> , <u>T846C (S95L)</u>	37
E7	INS668GGA, *T789C, *T795G, T843C	A645C (L28F), A647G (N29S), *T732C (F57V), <u>T846C (S95L)</u>	37
E7	G585A, T756C, *T789C, *T795G, C854DEL	A619T (T20S), G709A (A50T), <u>C712T (H51Y)</u> , * <u>T732C (F57V)</u> , C747DEL (Frame shift), A826DEL (Frame shift), A826T (I89F), <u>T844DEL (Frame shift)</u> , A852DEL (Frame shift, K97N, P98H)	37
Central India			
E6	A229G	GAT114TAC (D4Y), A225G (E41G), C240G (A46G), T244G (F47V), *C315G (S71C), A334T (R77S), *T350G (L83V), T400G (L99V), C422A (Q107K), C523A (C140Stop), G545A (E148K)	34
E7	C834T, C836A	T573G (D4E), *G823C (G88R), *T838C (I93T), *T841A (C94S)	34
East, Central and North India			
L1	T5675C, *G5698A, A5752C, A5755C, C5864T, *T6247C, A6316G, T6319C, C6370T, A6391G, A6425C, C6541A, C6559T, A6565G, T6661C, A6667C, G6721A, C6826T, *C6854T, C6865T, T6889G, A6952G, C6970T, G6994A, T7027G, G7031T, G7032A, G7060A, A7072T, T7078C, T7123C, A7141C	G6069C (R144T), *C6165A (T176N), G6173A (A179T), A6180C (N181T), C6182G (P182A), *A6434G (T266A), T6438G (V267G), A6445C (E269D), G6458A (D274N), A6492T (N285I), G6582A (R315Q), T6689G (S351A), *A6695C (T353P), A6803T (T389S), T7028G (L464V), *G7060T (L474F), A7148C (K504Q)	36
L2	A4242G, A4266C, T4281C, A4299C, A4410G, A4413C, G4428T, T4446G, T4452C, G4461A, A4467C, A4468C, A4504C, C4525T, T4527C, T4545G, G4563A/T, T4572G, A4599C, A4626T, A4632C, T4644A, T4647G, T4695C, *C4725T, A4788C, C4848T, A4887G, G4938A, A4944G, A4950G, A5034T, A5073T, G5142A, A5187C, T5286A, G5379A, T5403C/G, A5409G, T5412G, T5475A, T5478C, C5487T, A5517G, T5523C, A5532G, A5622G	C4253T (S6F), A4368C (Q44H), T4600C (S122P), G4621A (D129N), G4654A (D140N), A4693C (T153P), A4811C (N192T), A4820G (E195S), A4821C (I196L), C4825T & C4826A (P197Y), A4969G (T245A), G5008A (E258K), A5027C (N264T), T5041C (S269P), C5042A (S269Y), A5048C (N271H), A5059G (I275V), T5060A (I275N), A5063C (N276T), A5152C (I306L), *A5226T/C (L330F/F), C5231G (T332S), G5236C/A (D334H/N), A5250C (E338D), A5256T (E340D), A5356C (I374L), T5357G (I374S), T5368G & C5369T (S378V), T5386G (S384A), G5389A (V385I), A5466C (L410F), A5492C (I418T), T5495C (I420T), A5505C (Q423H), G5506A (A424T), C5509T (P425S), A5518C (I428L), C5564G (A443G), G5609A (R458Q)	36

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Genomic regions	Nucleotide variations/amino acid substitutions		References
	Synonymous variations	Non-synonymous variations (amino acid substitutions)	
L2 UTR/ NCR2	A4150C, C4151A, *T4152G/A, A4157G, T4158A, *C4164T, A4184T, T4186A, T4222C, *T4228C/G/A, T4231G, A4233C, A4234C		36
E1	T921C, T1221C, T1446C, G1941A, T2301C, T2343C, C2344T, T2470C, T2478C, T2778C	C874T (P4S), G1293T (Q143H), T1297A (L145I), G1363A (G167S), T1421C (I186T), A1842G (I326M), T1920G/C (D352E/D), G2337A (M491I), G2650A (E596K)	39
E2	T2914C, C3007T, G3058A, A3187C, A3253G, T3259C, T3313G, C3436A, A3538C, T3640C, T3664C, *C3684A, T3694A, T3706C, G3778T/C, C3787A, C3800G, T3805G, T3820C, T3847C	A2919C (N55T), C2988T (T78M), C3159A (T135K), C3161T (H136Y), T3172G (I139M), A3181C (E142D), G3182A (A143T), A3208C (Q151H), *T3224A (L157I), C3236G (H161D), G3249A (R165Q), A3285C (K177T), C3348T (S198F), A3362G (N203D), A3366C (E204A), T3371C (S206P), C3372A (S206Y), C3377G (P208A), T3387C (I211T), *C3410T (P219S), G3413A (A220T), C3516A (T254N), T3517C (T254N), T3566G (F271V), C3576T (S274L), G3585C (G277A), A3605G (N284D)	36
E4	A3362G, T3371C, C3377G, T3387C, *C3410T, G3413A, A3605G	A3366C (K9Q), C3372A (P11T), C3436A (S32stop), C3516A (L59T), T3517C (L59T), A3538C (Q66P), T3566G (H75Q), C3576T (H79Y), G3585C (D82H)	36
LCR	A7168G, T7176G, T7187C, G7193T, A7197C, A7227C, *A7233C, A7266G/C, A7376T, C7394T, C7395T, T7401C, G7427A, G7435A, *T7450C, A7482G, A7483C, *A7485C, *G7489A, *G7521A, A7550G, C7577T, C7655A, *C7669T, *C7689A, T7713G, T7714G/A, A7729C, T7743G, C7764T, C7792T, A7773C, T7775A, C7786T, G7826A, T7827A, G7834T, G7868A, C7886G, C13T, C24T, A93T, A98C		36
West and South India			
L1	*C5990T, *C5695T	<u>A4941C (N56T)</u> , C5000T (H76Y), <u>A5049C (N92T)</u> , <u>A5248C (L158F)</u> , T5307G (V178G), <u>A5316C/T (N181T/I)</u> , <u>A5481C (K236T)</u> , <u>A6101C (K443Q)</u> , <u>A6135C (K454T)</u> , <u>A6198G (K475R)</u> , C5301A (T176N), G5309A (A179T), *A5570G (T266A), A5628C (N285T), T5662A (S296R), *A5831C (T353P), A5939T (T389S), T5960C (S396P) and *G6196A/T (L474F)	40
L2		(L75F), (T85A), (T94A), (S122P/A), (S134R), (T245A), (L266F), *(S269P), (S270N), (D272N), (N273S), (I306L), *(L330F), (T332S), *(D334N), (D334H), (D334T), (E338D), (Q342L), (T352P), (H354Q), (T377S), (S378V/F), (S384A), (V385I), (L390F), (I418M), (I420T), (Q423H), (A424T), (S426A), (I428L), (A443G)	42
LCR	*G6657A, *T6586C, *A6865C, <u>A6475T</u> , <u>T6554C</u> , <u>G6606T</u> , <u>C6701T</u> , <u>A6737T</u> , <u>A6771T</u> , <u>C6805G</u> , <u>T6836G</u> , <u>T6847G</u> , <u>A6866C</u> , <u>T6917C</u> , <u>T6926A</u> , <u>T6967A</u> , <u>A6973C</u> , <u>A6975G</u> , <u>G6978A</u> , <u>G6988A</u> , <u>G6992A</u> , <u>G7004C</u> , <u>G7005A</u> , <u>C7012A</u> , <u>T7015C</u> , <u>C7025T</u> , <u>C7055T</u> , <u>C7066T</u> , C6530T, C6531T, A6618G, A6621C, G6625A, T6704G, A6772C, C6805T/G, C6825A, T6849G, T6850G, T6879G, C6900T, C6922T, C6928T, G6962A, G6970T, G7004A, C7022G		41
South and East India			
E6	A532G, T286A, A289G, <u>T421G</u> , <u>G522C</u>	*T350G (L83V), C335T (H78Y), <u>A276C (N58S)</u>	35

*Represents frequently observed variations. The underlined nucleotide variations represent the novel variations found in respective studies

Variants in West India: A study reported the prevalence of the European T350G variant in participants having coinfections with other HPV types, while the North American variant was observed only in women with coinfections with HPV16 and 33, suggesting that these variants may be facilitating coinfections with other

HPV types²⁵. In addition, 3 novel variations were observed. Phylogenetically, it was reported that the European T350G variant was the most predominant variant, followed by the European prototype (A1-3), North American (D1), and novel variants, based on the classification given by Yamada *et al*²⁶.

Table IV. Distribution of HPV16 lineages and sublineages among participants

Gene (no. of participants)	Variant lineages and sublineages	Percentage prevalence; % (sample type)	References
East India			
E2 (49 cases and 23 controls)	European (A1-3)	87.76 (cases) 96.3 (controls)	23
	Asian American (D2-3)	12.24 (cases) 3.7 (controls)	
LCR (42 cases and 19 controls)	European (A1-3)	88.09 (cases) 94.73 (controls)	
	Asian American (D2-3)	11.9 (cases) 5.26 (controls)	
Whole genome (145 cases and 24 controls)	European (A1)	86.89 (cases) 100 (controls)	24
	North American (D1)	4.13 (cases)	
	Asian American (D2)	8.96 (cases)	
West India			
E6 (32 single infections; 20 coinfection and 10 multiple types infections)	European (A1-3)	90.3	25
	North American (D1)	4.8	
	Novel variants	4.8	
North India			
L1 (31 cervical cancer patients)	European (A1-3)	70.9	27
	Asian American (D2-3)	12.9	
	North American (D1)	6.5	
	Novel variants	9.7	
E6 (60 cervical cancer patients)	European (A1-3)	85	
	North American (D1)	10	
	Asian American (D2-3)	3.3	
	New variants	1.7	
E7 (60 cervical cancer patients)	European (A1-3)	86.7	
	African-2 (C)	11.6	
	Asian (A4)	1.7	
LCR (60 cervical cancer patients)	European (A1-3)	63.3	
	Asian American (D2-3)	13.3	
	African-1 (B)	11.6	
	African-2 (C)	3.3	
	Novel variants	8.3	
E6 (74 cervical cancer; 9 SILs; 9 controls)	European (A1-3)	86.4 (cases) 100 (SIL) 88.9 (controls)	28
	Asian American (D2-3)	10.8 (cases) 11.11 (controls)	
	Asian (A4)	1.35 (cases)	
	African-2 (C)	1.35 (cases)	
E6 (221 cervical cancer cases)	European (A1-3)	97.25	30
	Asian (A4)	1.5	
	Asian American (D2-3)	0.75	

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Gene (no. of participants)	Variant lineages and sublineages	Percentage prevalence; % (sample type)	References				
South India							
L1 (57 samples)	European (A1-3)	48	33				
CENTRAL INDIA							
E6, E7 (21 cervical cancer cases)	European (A1-3)	52.38	34				
	Asian (A4)	47.61					
Multiregional							
E6 (50 cases and 20 controls)	European (A1-3)	92% (cases) & 100% (controls)	35				
	North American (D1)	4					
	Asian American (D2-3)	4					
E6, E7, and L1 (412 cervical cancer patients; Delhi-84 Mumbai-79 Kolkata-54 Bangalore-69 Thiruvananthapuram-59 Vellore-67)	European (A1-3)	86.8 (Delhi- 86.9 Mumbai-83.54 Kolkata-85.18 Bangalore-89.85 Thiruvananthapuram-81.35 Vellore-94.02)	37				
	Asian American (D2-3)	11.4 (Delhi-10.7 Mumbai-16.4 Kolkata-14.8 Bangalore-7.2 Thiruvananthapuram-13.6 Vellore-6)					
		Asian (A4)		1.7 (Delhi-2.38 Mumbai-0 Kolkata-0 Bangalore-2.9 Thiruvananthapuram-5.1 Vellore-0)			
				E1 (70 samples)	European (A1-3)	81.4	39
					Asian American (D2-3)	18.6	
				L1 (133 samples)	A	86.96	40
		European (A1)			48.1		
	European (A2)	26.3					
	European (A3)	0.75					
	Asian (A4)	9.8					
D	15.03						
North American1 (D1)	1.5						
Asian American1 (D3)	13.5						
LCR (as per L1 gene) (84 isolates)	European and/or Asian (A)	84.5	41				
	North American and/or Asian American (D)	15.5					
L2 (as per L1) (124 isolates)	European and/or Asian (A)	86.3	42				
	North American and/or Asian American (D)	13.7					

Table V. HPV18 nucleotide variations as well as lineage distribution found in different genomic regions

Region	Nucleotide variations		Lineage	Ref
	Synonymous variations	Non-synonymous variations		
E6/E7 gene variations				
North India	A89C, A92G, T104C, T116C, A218T, *T485C, A536C, *C549A, C751T	T553G, T858G	European (A1-3)	28

*Represents frequently observed variations. Ref, references

Variants in North India: A study analyzed the partial L1 and entire E6, E7, and LCR sequence variations among participants²⁷. Of 13 L1 variations, the most prevalent variation was A6695C (54.5%). Four novel variants were also observed. Furthermore, novel L1 variants with A6667C and A6691G variations did not vary within E6, E7, and LCR regions. The authors classified identified variants based on the classification given by Yamada *et al*²⁶. Most of the L1 variants belonged to the European (A1-3) lineage. Furthermore, 42 patients showed variations in the E6 gene. T350G variant was predominantly found in all the cases, either alone (81%) or with other variations (19%). Additional variations were observed only in variants consisting of T350G variation. Most of the E6 variants belonged to the European (A1-3) lineage. Additionally, five E7 variations were found only in eight cases, with the majority belonging to the European (A1-3) lineage. Furthermore, 45 cases comprised variations within the LCR region. The most frequent variation was G7521A (91.1%), followed by C7764T (22.2%) and T7714G (20%), mostly found along with G7521A. Four novel variants were observed in participants. Predominant lineages as per LCR were European (A1-3), followed by Asian American (D2-3), African-1(B), and African-2 (C).

Another study determined variations within E6 and E7 genes²⁸. Fourteen variations within the E6 gene were observed, of which four were novel in the Indian subcontinent and were observed exclusively among cases, indicating that these may be associated with oncogenicity. In addition, seven variations were

observed within the E7 gene, of which two were novel. The majority of the variants, as per the classification²⁹, were reported to belong to European (A1-3) lineage followed by Asian American (D2-3).

Additionally, another study reported six variations in the E6 gene³⁰. T350G (67.5%) variation was predominant, followed by C158T (15%) and G145T, T286A, and C335T (12.5%, each). As stated by the authors, European (A1-3) variants were found to be predominant.

Variants in South India: A study reported variations within E6 and E7 genes³¹. Six variants of the E6 gene were identified. Two variants had European prototype gene sequences, *i.e.*, 350T (9.1%) and T350G variant (19.6%), respectively. Furthermore, another variant (14.7%) had two variations, *i.e.*, T350G and C335T. The fourth variant (19.6%) had variations at T350G along with G145T. The fifth variant (28.7%) had variations, T350G, C335T, and G145T. The sixth variant (8.4%) observed had a variation of T419G as well as T350G. Additionally, some variants were found to be more predominant in CC patients than in controls, suggesting their association with the disease. It was noted that co-variations play an important role in determining the pathogenicity of a variant. Furthermore, one variant was prevalent in younger age group patients, *i.e.*, below 45 yr, pointing towards its aggressive nature. In addition, four variants of E7 were found in this study. One variant had variation at A647G (37.8%). Other variants had variations T732C (21.7%), C790T (23%), and T795G (17.5%), respectively.

Another study stated that 38 samples with intact E2 genes were used for variant analysis³². Variants were divided into four groups based on cleavage by different restriction endonucleases. Sequencing of four samples from each restriction fragment length polymorphism (RFLP) group showed that four isolates from group one did not have any variation, while 10 isolates had variation A2983G. The third group comprised 11 samples with 13 variations. Thirteen samples from the fourth group comprised 12 variations.

Similarly, the distribution of HPV types was determined among cervical samples from non-malignant, pre-malignant (LSIL and HSIL) and malignant samples (squamous and adenocarcinoma)³³. Sequence variations were analyzed among HPV16-positive samples within the L1 gene. Nucleotide variations involved deletions and insertions as well as single nucleotide polymorphisms (SNPs). Some

variations at 6726, 6729, 6730, 6732, 6738, 6739, 6741, 6742, 6744, 6759, and 6760 were found to be predominant among patients with single and multiple HPV infections. Phylogenetic analysis of 57 HPV16 positive samples along with 12 reference sequences led to the clustering of samples into three groups comprising 48.5, 29, and 18.9 per cent of samples, respectively. In addition, as per alignment with reference sequences with known lineages, 48 per cent of samples were reported to be aligned to the European (A1-3) lineage.

Variants in Central India: A study reported 12 variations within the E6 gene³⁴. The most prevalent variation was T350G (76.2%), followed by C315G (28.6%). In addition, six variations within the E7 gene of eight HPV16 isolates were observed. The most common variation was G823C (23.8%), followed by C838A and T841A (9.5%, each). The variants were stated to belong to European (A1-3) and Asian (A4) lineage.

Variants in multiple regions in India: A study observed eight variations within the E6 gene among 38 patients³⁵. T350G was the predominant variant in cervical neoplasia compared to controls. Three novel variations were reported. Only three controls harboured variations within the E6 gene. The variants, as per the classification²⁶, were reported to belong to European (A1-3), Asian American (D2-3) and North American (D1) lineage.

Another group stated 49 variations within the L1 gene of HPV16³⁶. The most common variations observed were G7060T with minor allele frequency (MAF) of 0.84, followed by A6434G and C6854T (MAF=0.16, each) and G5698A, C6165A, T6247C, A6695C, (MAF=0.14, each). In addition, there were 92 variations within the L2 gene. Non-synonymous variations in the L2 region were higher among CC cases. Common variations within the coding region were A5226T (MAF=0.64), A5226C (MAF=0.29), and C4725T (MAF=0.16). Furthermore, 48 variations were observed within E2 and E4 genes. The most common variations observed were C3410T (MAF=0.17), C3684A (MAF=0.16) and T3224A (MAF=0.14). In addition, 45 variations within LCR were observed. Predominant variations observed were T7450C (MAF=0.45), followed by G7521A (MAF=0.16) and A7233C, A7485C, G7489A, C7669T and C7689A (MAF=0.13, each). Additionally, 13 variations within L2-UTR or NCR2 were reported, with certain common

variations such as T4152G (MAF=0.97), T4228C (MAF=0.76), and C4164T (MAF=0.15).

A multicentric study reported 204 variations within the L1 gene of HPV16³⁷. Common non-synonymous variations were C6163A (13.9%), A6693C (12.4%), C5562G (12.2%), C5862T (12.2%), A6178C (9.7%) and G7058T (11.3%). However, common synonymous variations were C6852T (14.3%), A6665C (13.8%), G6992A (13.06%), and C6557T (12.6%). Phylogenetically, European (A1-3), Asian American (D2-3) and Asian (A4) lineages were reported, as per the given classification³⁸. Asian lineage was found only in Delhi, Bangalore, and Thiruvananthapuram samples. In addition, 56 variations were observed within the E6 gene. The most common non-synonymous variations were T350G (72.3%), followed by G145T (13.1%) and C335T (12.1%). Similarly, frequent synonymous variations were T286A (12.1%), A289G (11.7%), and A532G (8.3%). Five novel variations were also observed. Furthermore, 29 E7 gene variations were observed. Commonly observed variations were T789C (11.9%), T795G (11.9%), and T732C (9%). Additionally, six novel variations were observed.

Another study has reported variations within the E1 gene of the HPV16 genome³⁹. Out of 20 variations reported, G1293T (MAF=0.37), T1421C (MAF=0.21) and T921C, A1842G, C2344T (MAF=0.13, each) were commonly observed. Phylogenetic analysis, as per the previous study²³, demonstrated that among episomal E2 isolates, European (A1-3) and Asian American (D2-3) lineages were found. In addition, it was reported that variations within the NCR-2 region were higher among E2 intact cases as compared to controls. No microRNA (miRNA) binding site loss was observed among Asian American (D2-3) variants. Furthermore, no mRNA and protein expression of L2 was observed among cases harbouring integrated viral genome.

Another study observed 61 nucleotide variations within the L1 region in patients as well as controls⁴⁰. The most common variations observed were A5570G (73.3%), G6196A/T (26.3%), C5990T (17.1%), C5695T (15.1%), and A5831C (15.1%). In addition, certain variations were reported for the first time in the Indian population. In addition, L1 variations A5316C/T, A5831C, and A5939T, as well as variants belonging to Asian American1 (D3) lineage, were associated with HSIL. Phylogeny was determined based on the L1 gene, along with certain reference sequences with known lineages, and it was found that 19 L1 sequences were incomplete and, therefore, their phylogeny could

not be determined. Out of the remaining samples with complete L1 gene, 113 samples clustered with members of lineage A (European and/or Asian). However, 20 samples clustered with members of D (North American and/or Asian American) lineage. However, it was suggested that the entire genome should be used to ensure the phylogenetic distribution of variants.

Furthermore, another study observed 47 variations within LCR in studied samples, including 23 non-synonymous and 24 synonymous variations⁴¹. These variations were present in 64.8 per cent of samples with a normal cervix, 88 per cent of women with LSIL, and 89.8 per cent of women with HSIL. The most common variations were G6657A (69.3%), T6586C (27%), and A6865C (18.4%). Certain novel variations were also observed in Indian women. Furthermore, T6586C, G6657A, and T6850G variations were associated with HSIL. In addition, multiple variations were common in LCR from women having HSIL. Phylogenetically, the Asian (A4) sub-lineage was reported to have multiple variations related to HSIL. The phylogeny of variants was determined based on the L1 gene and by using reference sequences with known lineages. The corresponding L1 sequence of 18 isolates was not available, and thus, lineage could not be assigned. Among the remaining isolates, 84 clustered as per assigned lineages.

Another study observed 91 variations within the L2 gene, of which 43 were non-synonymous and 48 were synonymous⁴². These variations further translated to 35 non-synonymous substitutions and 53 synonymous substitutions. Substitutions L330F (75.6%), S269P (28.4%), and D334N (24.3%) were commonly observed. Sixteen substitutions reported in Indian isolates were novel. It was also reported that samples with multiple substitutions were associated with HSIL. Additionally, substitutions S384A, L266F, S378V, and T245A were also associated with HSIL and were further reported to be predominant in Asian American (D3) sub-lineage, substantiating its aggressive nature. Furthermore, substitutions T245A, L266F, S378V, and S384A were reported co-mutating in 19 samples. In this study, lineages were assigned to 134 of the total samples based on the L1 gene; 14 isolates without corresponding L1 gene were not assigned lineage. These 14 isolates, however, clustered with lineages A (European and/or Asian) and D (North American and/or Asian American) equally. Of the isolates with assigned lineage, 124 clustered as per assigned lineage. However, there was no difference in the predominance

of non-synonymous variations between normal and HSIL cervical statuses, as reported by Bhattacharjee *et al*³⁶.

HPV 18 variants: A study²⁸ from North India reported variations within the E6 and E7 genes of HPV 18. The study stated that all of these variants belonged to the European (A1-3) class, as per the classification by Ong *et al*⁴³. Common E6 and E7 variations observed were T485C and C549A among 13 samples.

Putative functional significance of HPV variants: Nine non-synonymous variations in L1 protein were observed in immune-dominant loops within L1 protein⁴⁰. Many of the variations were predicted to affect B-cell and /or T-cell binding, impairing the human leukocyte antigen (HLA)-interaction, while others were impacting the stability of 3D protein structure as well as L1 monomer and pentamer (Table VI)^{37,40}. There were 29 deleterious non-synonymous variations within the HPV16 genome, among which 26 were exclusively present within CC cases, while three were also present in controls³⁶. Among these deleterious variations, 17 were present within L1, L2, and E2 genes. Variations impacting B/T- cell epitopes were also observed in L2 gene⁴².

Similarly, the variations observed in the E6 protein were reported to affect B-cell epitopes, stability of the E6AP complex, and binding affinity of peptides to Major histocompatibility complex (MHC). In contrast, some variations were reported to create additional epitopes for HLA^{5,30}. The amino acid variations within the epitope regions may have altered immune response, and thus, the vaccines designed for a particular variant may have reduced efficacy against the variants found in other regions. Furthermore, 3D modelling predicted that variants with C523A variation within the E6 lack the last 12 amino acids of the second domain, which made the hook-like structure and interact with amino residues of the preceding domain³⁴. Thus, further studies must be conducted to evaluate the epitopes that can be affected by such variations for the rational design of HPV vaccines.

The significance of LCR variations was determined by their localization in various Transcription factor (TF) binding sites. Several variations were found in known binding sites of various TFs^{23,26,41}. These variations in the binding sites of various TFs were also reported to be associated with particular lineages. In addition, it was also reported that NCR-2 of HPV16 consists of 14 binding sites for miRNA³⁹. European variants, among

Table VI. Localization as well as functional implications of HPV16 genomic variations reported

Gene	Substitutions	Location/Function
L1	N56T, N92T, L158F, T176N, V178G, A179T, N181T/I, N285T, S396P, K454T	B-cell epitope
L1	H76Y, T266A, N285T, S296R	T-cell epitope
L1	L158F, A179T, K236T, T266A, S296R, T353P, S396P, K454T	Destabilize the 3D structure of the protein
L1	N56T, H76Y, N92T, T176N, V178G, N181T/I, N285T, T389S, K443Q, L474F	Stabilize L1 monomer
L1	T266A, T353P	Affects L1 pentamer stability
L2	L75F, T85A, T94A, T254A	B-cell epitope
L2	T85A, S122P/A, S134R, T245A, L266F, S269P, S270N, I306L	T-cell epitope
E6	F69L, M137L	B-cell epitope
E6	H78Y, L83V	Affect epitopes of MHC I & II Creates additional HLA epitopes
E6	L83V+F69L L83V+M137L	Destabilizes E6AP complex
LCR	G7521A, C7786T	YY1 and YY14 binding site
LCR	T7714G, T7743G, C7764T	NF1 binding site
LCR	G7004A/C, G7868A	E2 binding site

MHC, major histocompatibility complex; HLA, human leukocyte antigen; E6AP, E6 associated protein; LCR, long control region

those having intact genome, were reported to have T4228C variation, which was linked with the loss of nine miRNA binding sites.

Discussion

The viral early genes are associated with transformation and oncogenicity, while the late genes are related to capsid formation and the non-coding regions perform regulatory function^{6,36,44}. Hence, any variation within HPV genes may affect its transformational potential.

Studies have reported that different variants of HPV have varying oncogenic potential and risk associations. For instance, non-European variants of HPV16 are associated with an increased risk of persistence, cervical intraepithelial neoplasia (CIN), and cancer development¹³. In addition, these variants have also been reported to be associated with histologic subtypes.

Upon analyzing the results of the phylogeny of variants from the given studies, it was found that the distribution of lineages varies with the different genomic regions sequenced. However, all such studies have relied on sequencing specific genes of the viral genomes, such as L1, E2, E6, E5, LCR, *etc.*, either singly or in combinations of two or more genes, rather than whole viral genome sequencing. Thus, it is required to sequence the complete genome to ascertain

the specific lineage of the variants. Additionally, the risk of variants varies with the ethnicity of the infected population, as Asian women infected with the A4 sublineage and Hispanic women with D2/3 sublineages were reported to have a higher risk of CIN3+ compared to other ethnic groups⁴⁵. Although nucleotide variations within a particular gene might be associated with disease susceptibility, a haplotype comprising such variation might not depict significant association, thus posing difficulty in ascertaining risk association and selecting biomarkers³⁶.

A critical analysis of all these studies indicated that there are certain unique as well as common variations in the HPV genome with respect to geographical regions (Fig. 2A-C). Here, the studies in which there was no segregation of patients from different regions, as well as those genes for which less than four geographical regions were represented, were excluded from the analysis. Furthermore, it can be noted that there are more exclusive variations in southern India as compared to other regions, which could be attributed to the sample size as well as the inclusion of more than one city in the southern region. Thus, suggesting that more studies need to be done in this direction from other regions of India.

In addition, there were certain variations within the HPV16 genomes that were common in different regions (Fig. 3A-C); however, their prevalence varies

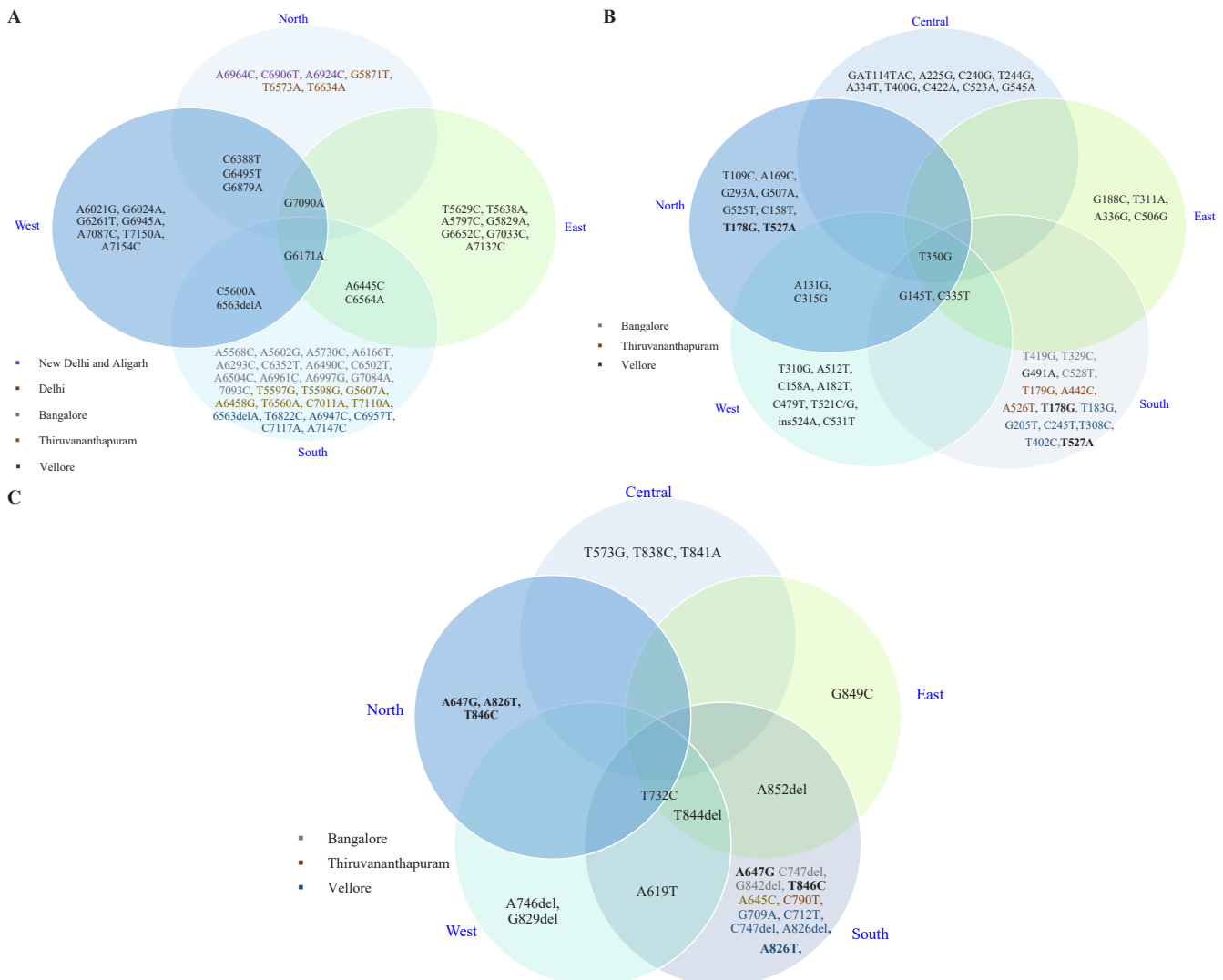


Fig. 2. Distribution of HPV16 non-synonymous variations within different regions of India (A) L1 variations, (B) E6 variations, and (C) E7 variations (different colors in text represents different locations from the same region).

among them. For instance, C3161T, T3517C, T3566G, and T3694A variations in the E2 region were relatively more prevalent in south India²⁹, and C3684A was more frequent in east India²³. The differences in variation patterns observed in various genomic regions may be attributed to regional characteristics, ethnicity of the population as well as geographical conditions. Keeping in view HPV variants and their antigenicity, it has been reported that amino acid substitutions within certain HPV variants may affect the immune response and thus, vaccines developed against one variant may have reduced efficacy in regions where these variants are less prevalent⁷. Furthermore, a study was designed to assess cross-protection offered by HPV Bivalent/Quadrivalent vaccine towards variants of genetically related hr-HPV infections^{16,45}. It was demonstrated

that the vaccine-induced antibodies depicted reduced neutralization of HPV 45 (B2 variants)¹⁶. Similarly, the vaccine efficacy against HPV 31 lineage A/B was partial for transient infections and not efficacious for persistent infections⁴⁵. While, in other studies, altered neutralization against HPV 33 (A2, A3, B, and C variants), HPV 52 (D variants), and HPV 58 (C variants) was observed¹⁷⁻¹⁹. This variability in neutralization was observed to occur because of specific residues located within the immune dominant loops of the L1 protein. In contrast to the extensive studies available for some other viruses^{46,47}, limited evidence is available regarding vaccine escape of the HPV variants. Therefore, additional studies should be carried out to evaluate the impact of HPV genomic variations within the epitope region of various genes

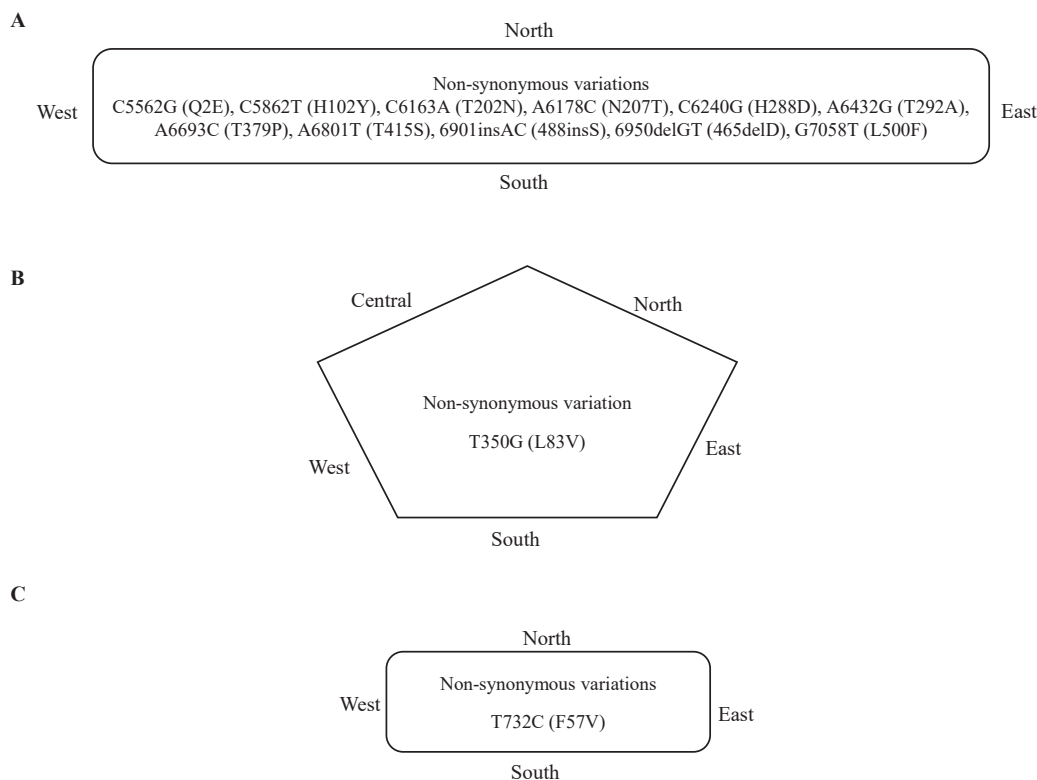


Fig. 3. Common variations found in different regions of India (A) L1 variations, (B) E6 variations, and (C) E7 variations.

for rational vaccine design. In addition, the biological significance of the variants with such variations needs to be validated through experimental studies.

Conclusion

The present compilation highlights some significant findings, including regional variations in HPV variants and their putative functional significance. Comprehensive studies encompassing participants from different regions and ethnicities in a large data set are required to ascertain clinically significant HPV variants. The majority of studies reported hitherto have focused on particular genes for variant identification rather than the entire viral genome. Therefore, further studies involving the whole viral genome approach are required to ascertain the distribution of variants from different geographical regions as well as for better identification of polygenic aspects of viral contribution to diseases. In addition to HPV-16 and -18, studies also indicate the involvement of other hr-HPV types, so while planning such studies involving variants detection, these HPV types should also be taken into consideration. This will help not only in better diagnosis and management but also in vaccine design for HPV. Keeping in consideration that HPV is

not only a menace for CC but many other oncogenic conditions, such studies should also be carried out in other relevant disease conditions.

Acknowledgment: First author (NS) acknowledges the Council of Scientific & Industrial Research for providing fellowship to pursue PhD research.

Financial support & sponsorship: None.

Conflicts of Interest: None.

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation: The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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