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Dengue Virus Type 2: Global Epidemiology, Molecular Evolution, and Immune Response Insights

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Abstract

Dengue fever is a globally prevalent arboviral disease caused by four serotypes of dengue virus (DENV), DENV−2 emerges as the most clinically consequential due to its expanding geographic spread and intensifying outbreaks worldwide, while its long-term evolutionary trajectories and genotype-specific transmission features remain insufficiently resolved. Through the integration of global surveillance data from 2014–2024 with complete genome sequences, this study delineates the spatiotemporal spread patterns of DENV−2, reconstructs phylogenetic relationships across its six genotypes, and characterizes key genomic variations, including amino acid substitutions, recombination signals, and divergence between structural and non-structural proteins. Our analysis further reveals a rising trend in global DENV−2 activity, accompanied by recurrent epidemics in Southeast Asia, South America and East Africa and recent autochthonous transmission in temperate Europe with this expansion fueled by climate variability, human mobility and vector adaptation. Phylogenetically, the cosmopolitan genotype dominates globally whereas Asian II and American lineages are geographically constrained, and the Asian/American recombinant lineage persists in parts of Asia and Latin America. Additionally, comparative genomic analyses highlight genotype-dependent mutation heterogeneity and thirteen validated T-cell epitopes enriched in non-structural regions, which underscore relevance to $CD8^+$ immunity. The review synthesizes critical insights into DENV−2 evolution, adaptation and immunogenicity, emphasizing the need for strengthened genomic surveillance and prioritization of conserved targets for next-generation dengue vaccines.

1. Introduction

Dengue fever (DF), an acute viral infectious disease caused by the dengue virus (DENV), is predominantly prevalent in tropical and subtropical regions. It is widely recognized as the arboviral disease with the most extensive epidemic scope and the heaviest economic burden globally [1], which officially designated by the World Health Organization (WHO) as one of the top ten global health threats in 2019 [2]. Epidemiologically, *Aedes aegypti* and *Aedes albopictus* serve as the primary vectors for the transmission of DENV [3], and the acceleration of climate change and urbanization over recent decades has driven the continuous expansion of DF prevalence, thereby triggering a sharp increase in the global incidence of the disease. In 2024, the global number of DF cases surpassed 7.6 million across more than 90 countries and regions [4, 5], while many dengue-endemic nations in tropical and subtropical zones have reported record-high numbers of cases alongside mortality rates exceeding normal levels [6]. Furthermore, the emergence of small-scale DF infections in several European countries and regions [2], ultimately resulting in half of the world's population being exposed to the threat of DENV infection.

DENV is a single-stranded positive-sense RNA virus belonging to the genus *Flavivirus* within the family *Flaviviridae*, and it is classified into four distinct serotypes (DENV−1, DENV−2, DENV−3, and DENV−4) based on the antigenic differences in its envelope protein (E protein). The genome of DENV is approximately 10.6–11 kb in length with a molecular weight of 4.2×10^6, which consists of a linear non-segmented positive-sense RNA strand that contains a single long open reading frame (ORF) flanked by two untranslated regions (5'-UTR and 3'-UTR) [7, 8], notably, it possesses a type 1 cap structure (m7GpppAmpN2) at the 5'-UTR end and lacks a poly(A) tail at the 3'-UTR end [9, 10]. The polyprotein translated by viral RNA is ultimately cleaved by viral NS3 and host protease into three structural proteins (capsid (C), membrane (M), envelope(E)), and seven non structural proteins (NS)(NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5) [11]. The four serotypes of DENV differ from each other by 25%−40% in amino acid levels and are further divided into genotypes that exhibit genetic differences of up to about 3% [12]. Infection with one serotype can confer long-term immunity against that same serotype, but only transient or partial immunity against other serotypes, and re-infection with a heterologous serotype increases the risk of developing severe dengue, a phenomenon referred to as antibody-dependent enhancement (ADE) [13, 14]. DF is a self-limiting disease that is usually asymptomatic or causes mild febrile illness though some cases may develop into severe dengue shock syndrome (DSS) and dengue hemorrhagic fever (DHF).

DENV−2 is the most pathogenic among the four DENV serotypes as its severe case rate and mortality are significantly higher than those of the other serotypes and it is linked to a higher proportion of severe infections during secondary infections when compared with DENV−1 and DENV−4 [15]. Notably DENV−2 maintains an extensive global distribution across tropical and subtropical regions where endemicity is particularly high in areas such as Southeast Asia, South America and East Africa thus underscoring its worldwide epidemiological significance [16]. The first documented reports about DENV−2 date back to 1944 when the virus was identified in Papua New Guinea and Indonesia, and this was followed by sustained reports of DENV−2 circulation in Nigeria and Puerto Rico during the 1960s [17]. In recent years multiple large-scale outbreaks of dengue fever caused by DENV−2 have occurred globally which include the severe 2017 outbreak in Sri Lanka that resulted in 186,101 infection [18] and the 2019 outbreak in Brazil that led to the reporting of 1,489,457 cases [19].

Current research on DENV−2 mostly focuses on specific regions or short-term epidemic dynamics and lacks systematic analyses at a global scale over the past decade. Consequently, the epidemiological significance of genotype-specific transregional transmission routes, key genomic variation sites, and recombination events remains incompletely elucidated. Herein, this study integrates global DENV−2 genomic data with epidemiological surveillance information from 2014 to 2024. It aims to clarify the global and regional epidemic trends and genotype distribution characteristics of DENV−2, explore patterns of genomic sequence variation, and analyze their implications for the virus's global transmission. Ultimately, we seeks to provide a scientific evidence base to support global DENV−2 epidemic early warning systems, foster cross-regional prevention and control collaboration, and inform the development of novel vaccines.

2. Materials and methods

2.1. DENV genome sequencing

The search that employed keywords such as "dengue virus type 2", "DENV−2 complete genome", and "Dengue virus 2 full-length genome" was conducted on July 24, 2024, in the NCBI GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) and had a scope that encompassed DENV−2 genome sequencing data from 2014 to 2024 worldwide. The inclusion criteria were defined as follows: studies must include explicit information regarding sampling time (accurate to the year), sampling location (accurate to the country/region), and host details (either humans or vector mosquitoes). low-quality sequences (with ambiguous base proportions < 5%) and redundant submitted sequences must have been removed through sequence quality control procedures. Following curation, a final dataset of 135 complete genome sequences was obtained, originating from global regions including Asia (China, India, Singapore, Thailand, Cambodia, etc.), Africa (Egypt, Tanzania, Mozambique, etc.), the Americas (the United States, Brazil, etc.), Europe and Oceania, and and collectively represented six recognized genotypes: Cosmopolitan, American, Asian I, Asian II, Asian/American, and Sylvatic.

2.2. Genomic characterization and phylogenetic analysis

Phylogenetic analysis was performed on the complete sequence using the Maximum Likelihood (ML) method. The optimal nucleotide substitution model was selected according to the Akaike Information Criterion (AIC) using ModelTest 3.7 software, which identified the TN93 + G + I model as best fitting the data [20]. The phylogenetic tree was constructed using the Molecular Evolutionary Genetics Analysis (MEGA) 7.0 software, with 1000 bootstrap replicates performed to assess node reliability.

The reference DENV−2 whole viral genome sequences used for constructing different phylogenetic branches were obtained from the GenBank database and are listed by country and accession number as follows: China (MH827529.1, MK783206.1, KC964094.1, MG840613.1, MT856317.1, MK783194.1, KU094070.1, KY672948.1, KX262927.1, KX262942.1, MT705582.1). Guadeloupe (OR229975.1, OR229976.1, OR229965.1, OR229973.1, OR229960.1, OR229962.1, OR229958.1, OR229964.1, OR229966.1, OR229968.1, OR229969.1, OR229970.1, OR229971.1, OR229972.1). Reunion (MZ130518.1, MZ130527.1, MN272404.1). India (MK858111.1, MK858107.1, OP310805.1, OQ339137.1, KY495802.1). Singapore (MW512468.1, MW512445.1, MW512449.1, HM067746.1, MT252652.1). Seychelles (MN272405.1). France (MN720746.1). Djibouti (MZ130528.1). Indonesia (GQ398263.1, AB189122.1, KY006142.1, KC762669.1, GQ398257.1). Australia (AY037116.1). Thailand (KU509272.1, KT806317.1, MZ520931.1, MZ636770.1, KT781538.1, DQ181801.1, DQ181799.1, KY586589.1, MN955664.1). Brunel (EU179857.1). Malaysia (KJ806785.1, EF105379.1). Egypt(MZ130522.1). Cambodia (OL414723.1, DQ518649.1, FJ639717.1, GU131897.1, GU131932.1, KY495819.1). Laos (MN444616.1, MN444619.1, KY849763.1). Bangladesh (MN328061.1, LC436655.1). Tanzania(MZ130526.1). Mozambique (MZ130519.1). Cameroon (MZ130525.1). Mauritania (MT980927.1, MT981085.1). Senegal (MT981011.1, MT981148.1, EF105389.1, EF105384.1). Burkina (MT982731.1, KY627763.1, MT982126.1, MT982148.1). Benin (MZ130524.1). Taiwan(HQ891024.1, KJ734727.1). Philippine (KF744400.1, KF744407.1, KF744404.1, AY786372.1). Vietnam (FM210209.1, FJ410217.1, FM210214.1). Venezuela (GQ868540.1, GQ868596.1). Brazil (JX669488.1, GQ868640.1, FJ850082.1, GU131884.1). Nicaragua (GQ199868.1, EU482600.1, EU482634.1). Belize (FJ898461.1). Colombia (FJ024477.1, GQ868592.1). Cuba (AY702036.1). Myanmar (DQ518653.1, KX357994.1, MW332624.1, MW788983.1, KT806324.1). Tonga (HM582117.1). American (HM582107.1). Mexico (GQ868589.1, AF100469.1, JX966379.1). Peru(AF100467.1, EU056811.1). Ibadan(DQ341197.1, EF105387.1, EU003591.1, EF105388.1, EF105385.1).

2.3. Recombination analysis

Recombination events and molecular evolutionary analysis were investigated using the RDP5.5 software package. The reference viral sequences selected for recombination analysis were strategically retrieved from the GenBank database with sequence selection based on two key criteria: phylogenetically representative as determined by preliminary phylogenetic tree construction, and geographically proximate to the target strains to minimize potential biases from evolutionary divergence or spatial separation. The selected reference sequences with their corresponding detailed annotations are as follows: KF744404.1, MW788983.1, OR229969.1, GQ199868.1, EU056812.1, EF105389.1, OL414723.1.

2.4. Secondary structure analysis of complete genome

To investigate the structural characteristics underlying the genetic divergence among DENV−2 strains, a systematic secondary structure analysis was performed on the complete polyprotein using PSIPRED v4.0 (https://bioinf.cs.ucl.ac.uk/psipred/). This widely recognized bioinformatics tool employs support vector machine (SVM) algorithms trained on multiple sequence alignments and profile Hidden Markov Models (HMMs) to predict three major secondary structure elements (α-helix, β-sheet, and random coil) with robust reliability in virological research [21].

Prior to structural prediction, the complete genomic sequences of the DENV−2 reference strain (GenBank accession number: M29095.1) and seven geographically distinct or epidemiologically representative strains (GenBank accession numbers: KF744404.1, MW788983.1, OR229969.1, GQ199868.1, EU056812.1, EF105389.1, OL414723.1) were retrieved from the GenBank. These sequences were translated in silico into full-length polyprotein amino acid sequences using the ExPASy Translate Tool to ensure format consistency [22]. Subsequently, pairwise comparative analyses of secondary structure profiles were conducted between the reference strain and each representative strain. Differences in the distribution, length, and relative abundance of α-helices, β-sheets, and random coils across the entire polyprotein were quantified. For the

evaluation of potential helical structures, specific parameters including helical propensity scores, predicted helical length (number of amino acid residues), conservation of helical regions across strains, and spatial localization within functional domains (e.g., enzymatic domains of non-structural proteins, structural domains of envelope protein) were analyzed to assess their potential functional relevance [23].

2.5. Characteristic analysis of DENV-2 MHC epitopes

The SYFPEITHI database (https://www.syfpeithi.de/), a well-established and peer-reviewed resource dedicated to curating MHC class I and class II ligands as well as peptide motifs with entries exclusively derived from published scientific studies and it currently houses over 7000 validated peptide sequences, was utilized to systematically retrieve MHC epitopes unambiguously annotated serotypes as "DENV−2" or "DENV−2 (NGC strain)" [24]. Subsequent bioinformatic characterization of these T cell-eliciting MHC epitopes which encompass both cytotoxic T lymphocyte (CTL) epitopes and helper T (Th) cell epitopes with characterization encompassing three key dimensions: First, the classification of their source proteins by functional type which distinguishes between structural proteins and non-structural proteins of DENV−2. Second, the mapping to precise positions within the amino acid sequence of the DENV−2 reference strain (M29095.1) to define the start and end residues of each epitope.

3. Results

3.1. Global epidemiological trends of DENV-2

Since 2014, the global incidence of DENV−2 infections has exhibited a consistent and statistically significant upward trajectory. This trend is closely associated with the synergistic effects of climate change, rapid urbanization, and the expansion of vector habitats, particularly within major endemic foci in Southeast Asia, South America, and East Africa, where DENV−2 has maintained high transmission intensity and established stable endemic cycles. The year 2023 marked a critical turning point with historic large-scale outbreaks reported in epidemiologically vulnerable countries such as Brazil, Bangladesh and the Philippines where confirmed case numbers surpassed one million in each region. This reflected an unprecedented epidemic intensity that imposed substantial burdens on local healthcare systems [5, 6].

Furthermore the occurrence of sustained autochthonous transmission events in temperate European regions, including France and Italy which were previously considered non-endemic for dengue viruses, signifies a potential northward expansion of DENV−2 endemic range. This indicates an emerging spatiotemporal spread trend into non-traditional temperate latitudes, a geographical expansion likely driven by rising global temperatures, increased human population mobility, and the adaptive capacity of vector species such as *Aedes aegypti* and *Aedes albopictus* to colonize temperate environments [2].

3.2. Phylogenetic analysis reveals genotype distribution

Phylogenetic analysis indicates that DENV−2 strains worldwide are classified into six distinct genotypes. Among these, the cosmopolitan (42.3%), Asian II (28.7%) and American (16.5%) genotypes are identified as the predominant lineages **(**Fig. 1**).** Over the past decade, the cosmopolitan genotype has emerged as the global dominant lineage which exhibits remarkable adaptability and superior transmission fitness. It is now widely distributed across Southeast Asia, South Asia, the Middle East, Africa, Pacific Islands, and South America. In contrast, the Asian I and Asian II genotypes remain largely confined to mainland Southeast Asia and certain Pacific islands. Whereas the prevalence of the American genotype has relatively declined in Latin America, the Asian/American recombinant genotype has gained increasing significance and serves as a major driver of outbreaks in parts of the Americas and Asia, such as Thailand and Vietnam [20].

3.3. Genomic variation and amino acid substitutions

Whole-genome alignment of prevalent strains from 2014–2024 with the reference strain (M29095.1) revealed an average nucleotide sequence identity of 91.5%–93.5%, corresponding to a variation rate of 6.5%–8.5%. Recombination events were predominantly detected within non-structural protein regions especially near the gene junctions of NS1-NS3 and NS5, whereas structural protein regions exhibited a considerably lower recombination frequency.

3.3.1. Amino acid mutations in structural protein regions

In the structural protein region of the seven analyzed virus strains, the nucleotide sequence encoding C-prM/M-E was 2322 nt in length which corresponds to 774 amino acids. Owing to the high conservation of the DENV−2 structural protein coding region, the nucleotide lengths of the three structural proteins were identical across all seven strains. The amino acid lengths for the capsid, precursor membrane and envelope proteins were 113, 166, and 495 residues, respectively thereby reflecting the strict conservation of structural protein architecture in DENV−2 strains. Compared to the DENV−2 reference strain M29095.1, the total numbers of base substitutions in the C-prM-E region were: 96 (KF744404.1), 140 (MW788983.1), 144 (OR229969.1), 119 (GQ199868.1), 176 (EU056812.1), 1008 (EF105389.1) and 147 (OL414723.1). The numbers of non-synonymous substitutions were: 14 (KF744404.1), 22 (MW788983.1), 20 (OR229969.1), 20 (GQ199868.1), 16 (EU056812.1), 368 (EF105389.1) and 21 (OL414723.1). Consequently, the non-synonymous mutation rates for the C-prM-E structural protein region were 14.6%, 15.7%, 13.9%, 16.8%, 9.1%, 36.5% and 14.3%, respectively (Table 1).

3.3.2. Amino acid mutations in non-structural protein regions

In the non-structural protein region, the NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5 sequences of the seven strains of viruses were highly consistent, with a length of about 7854nt. According to the sequence alignment results (Fig. 2), all strains harbored several single nucleotide variations in this region relative to the reference strain M29095.1, some of which induced amino acid substitutions. The variation of NS1 region was relatively concentrated as it contains roughly 60–75 single nucleotide mutations, including 7–11 non-synonymous substitutions. Additionally, numerous polarity or charge-altering amino acid changes (e.g., H→Y, S→L) were observed which may trigger minor conformational adjustments at random coil and hydrophobic interface domains.

The NS2A-NS2B region contained 70–85 base mutations, of which 10–15 were non-synonymous substitutions. Substitutions such as V→A and I→T were detected in the hydrophobic transmembrane region of most viral strains, whereas the NS3 region was relatively conservative yet harbored numerous amino acid substitutions within its protease and helicase functional domains (e.g., A→T, V→I, S→L). The NS4A and NS4B regions exhibited fewer base substitutions that numbered approximately 7–10 and 35–40 respectively. The NS5 region which is the longest and most conserved non-structural protein region possessed around 170–210 base substitutions, including 24–30 nonsynonymous substitutions that corresponded to a substitution rate of roughly 2.6%–4.3%.

Table 1
Comparison of amino acid and nucleotide substitutions in structural and non-structural proteins of seven DENV-2 strains.

strain		C	prM	E	NS1	NS2A	NS2B	NS3	NS4A	NS4B	NS5	Total
KF744404.1	Base substitution	11	14	71	44	22	19	99	21	25	91	417
	Base substitution rate(%)	3.245	2.811	4.781	4.167	3.364	4.872	5.34	4.667	3.36	3.37	4.1
	AA substitutions	1	4	9	10	4	3	9	3	2	9	54
	AA substitution rate(%)	0.885	2.41	1.818	2.841	1.835	2.308	1.456	2	0.806	1	1.593
MW788983.1	Base substitution	10	35	95	70	54	16	122	21	47	144	614
	Base substitution rate(%)	2.95	7.028	6.397	6.629	8.257	4.103	6.58	4.667	6.317	5.333	6.037
	AA substitutions	2	6	14	10	11	2	7	1	3	15	71
	AA substitution rate(%)	1.77	3.614	2.828	2.841	5.046	1.538	1.133	0.667	1.21	1.667	2.094
OR229969.1	Base substitution	21	33	90	76	54	22	135	40	51	186	708
	Base substitution rate(%)	6.195	6.627	6.061	7.197	8.257	5.641	7.282	8.889	6.855	6.889	6.962
	AA substitutions	2	6	12	7	7	2	10	4	4	21	75
	AA substitution rate(%)	1.77	3.614	2.424	1.989	3.211	1.538	1.618	2.667	1.613	2.333	2.212
GQ199868.1	Base substitution	20	24	75	59	47	25	127	27	52	157	613
	Base substitution rate(%)	5.9	4.819	5.051	5.587	7.187	6.41	6.85	6	6.989	5.815	6.028
	AA substitutions	4	5	11	2	9	1	17	2	6	23	80
	AA substitution rate(%)	3.54	3.012	2.222	0.568	4.128	0.769	2.751	1.333	2.419	2.556	2.36
EU056812.1	Base substitution	18	34	124	88	61	43	153	42	68	210	841
	Base substitution rate(%)	5.31	6.827	8.35	8.333	9.327	11.026	8.252	9.333	9.14	7.778	8.269
	AA substitutions	0	4	12	11	6	2	14	2	6	23	80
	AA substitution rate(%)	0	2.41	2.424	3.125	2.752	1.538	2.265	1.333	2.419	2.556	2.36
EF105389.1	Base substitution	39	100	264	187	148	82	340	84	134	491	1869
	Base substitution rate(%)	11.504	20.08	17.778	17.708	22.63	21.026	18.339	18.667	18.011	18.185	18.378

strain		C	prM	E	NS1	NS2A	NS2B	NS3	NS4A	NS4B	NS5	Total
	AA substitutions	5	10	31	24	35	4	30	8	12	62	221
	AA substitution rate(%)	4.425	6.024	6.263	6.818	16.055	3.077	4.854	5.333	4.839	6.889	6.519
OL414723.1	Base substitution	23	26	98	69	55	31	151	35	51	189	728
	Base substitution rate(%)	6.785	5.221	6.599	6.534	8.41	7.949	8.145	7.778	6.855	7	7.158
	AA substitutions	2	8	11	9	6	3	14	3	4	18	78
	AA substitution rate(%)	1.77	4.819	2.222	2.557	2.752	2.308	2.265	2	1.613	2	2.301

3.4. Possible secondary structures in structural and non-structural protein regions

Using the DENV−2 reference strain M29095.1 as a benchmark, predictive secondary structure analysis was performed for both structural and non-structural proteins of the seven representative strains. The results demonstrate high conservation of secondary structure within core functional domains, whereas variations in non-core regions exhibited genotype-specific divergence. Notably, the secondary structure discrepancies between the sylvatic strain (EF105389.1) and the human epidemic strains (Asian I, Asian II, cosmopolitan and Asian/American genotypes) were particularly pronounced.

3.4.1. Structural protein secondary structure conservation and variation

In structural proteins, specifically the dimerization domain of the C protein was dominated by α-helix (accounting for 60%–65%). The α-helix variation of this region among the seven genotype-representative strains was all < 3%, the fluctuation of random coils was < 2% and the proportion of β-sheets remained largely unchanged. For the transmembrane region of the prM protein the α-helix content of human epidemic strains was stably maintained at 75%–78%, showing < 2% discrepancy relative to M29095.1. In contrast, the sylvatic strain alone exhibited a reduction to 68%–70%, attributable to V→I and L→F mutations, yet it retained the ability to maintain viral envelope stability. The core functional domains of the E protein encompassed the fusion peptide and the receptor-binding domain. The α-helix content of the fusion peptide region in all human epidemic strains was 80%–82% which was fully consistent with the reference strain. The sylvatic strain displayed a decrease in α-helix proportion to 72%–74% owing to amino acid mutations and was hypothesized to mediate virus-host membrane fusion via functional compensation by amino acids in other regions. In contrast, the β-sheet content of the receptor-binding domain was stably maintained at 34%–36% in human epidemic strains and displayed a discrepancy of < 1% compared to the reference strain M29095.1, whereas only the sylvatic strain exhibited a reduction to 28% due to K→N mutation and its predicted receptor-binding interface area was diminished by 12.5% which ultimately impaired the host-binding function of the E protein in this strain (Fig. 3).

3.4.2. Non-structural protein secondary structure conservation and variation

In non-structural proteins, the secondary structures of the NS3 protease active site and the NS5 polymerase catalytic domain were fully conserved across all strains, whereas the NS2A exhibited the most prominent structural variation. The secondary structure parameters of the core conserved regions were highly consistent among all strains, as the NS3 protease active center and the NS5 polymerase/methyltransferase domains were 100% conserved with the fluctuation of α-helix and β-sheet proportions < 0.5% and the NS1 replication regulatory domain had an α-helix content of 35%±1% and a β-sheet content of 25%±1%. Although the random coil proportion of this region in EF105389.1 was increased by 2%, this alteration did not exert a significant impact on the viral replication process. In the genotype-specific variation regions, the difference in the NS2A protein was the most prominent as the α-helix content of EF105389.1 drastically decreased from 29% of the reference strain to 18% and the random coil proportion increased from 45% to 67%, suggesting that this variation may impair viral assembly efficiency. In sharp contrast, the secondary structure variation of the NS2A protein in all human epidemic strains was < 3% and was nearly consistent with the reference strain M29095.1 which further reflected the conservation of human epidemic strains in structures associated with viral replication regulation.

3.5. Characteristic analysis of DENV-2 MHC epitopes

Screening of the SYFPEITHI database for MHC epitopes unambiguously identified as "DENV−2" or "DENV−2 (NGC strain)" yielded a total of 13 epitopes derived from multiple viral proteins, including both structural and non-structural components (Table 2).

Among these 13 MHC epitopes, 4 (30.8%) were located within structural proteins and 9 (69.2%) were derived from non-structural proteins, indicating that T-cell epitopes of DENV−2 are predominantly located in non-structural proteins which may be linked to the key roles these proteins play in viral replication and immune evasion. Regarding specific target proteins, NS5 and the E protein each contained 3 epitopes which identifies them as the core proteins enriched for DENV−2 T-cell epitopes. In contrast, prM, NS2A, and NS4A each contained only a single epitope which suggests that these proteins possess relatively weaker immunogenicity.

Analysis of the peptide lengths revealed that 3 epitopes (23.1%) were 9 amino acids (aa) long, primarily originating from the structural proteins prM and E that 8 epitopes (61.5%) were 10 aa in length and were exclusively derived from non-structural proteins (NS1, NS2A, NS4A, NS4B, and NS5). And 2 epitopes (15.4%) were 15 aa long and were both located within the E protein. The average length of the identified MHC epitopes was 10.54 aa. This profile, dominated by short peptides (9–10 aa), was consistent with the characteristic of typical MHC class I-restricted epitopes thereby supporting their primary role in CD8 + T-cell responses.

Integration of the MHC epitopes' length and location with the DENV−2 proteome determined that the epitopes on the structural E protein were concentrated in the C-terminal domain III which was also known to react with convalescent patient serum. Combined with previous studies, this indicates that domain III contains both B cell and T cell epitopes, endowing it with diagnostic potential and value for vaccine development. Furthermore, the three epitopes identified in NS5 were clustered within the amino acid region 475–620, highlighting this region as a key candidate for DENV−2 cell-mediated immunity vaccine development.

Upon DENV−2 infection, antigen-presenting cells first engulf the virus, and its structural proteins and non-structural proteins are degraded into peptides, a process that is reflected in the viral genome structure, as shown in the figure. These peptides bind to MHC class I molecules and are presented on the surface of APCs. Subsequently, CD8 + T-cells recognize the peptide-MHC I complexes via their T cell receptor (TCR), and differentiate into effector cells through co stimulatory interactions and intracellular signaling cascades. These effector cells secrete cytokines such as IFN-γ and TNF-α or exerting cytolytic effects against infected cells. Regarding the association with ADE, peptides derived from structural proteins correspond to viral surface antigens, making them prone to interaction with sub-neutralizing antibodies and thus facilitates ADE. In contrast, peptides from non-structural proteins are not involved in ADE but instead mediate robust antiviral CD8 + T-cell responses, although NS1-derived peptides are associated with vascular pathological damage rather than ADE. Collectively, this differential involvement underscores the necessity of considering protein type when evaluating DENV−2 immunopathology and advancing vaccine development (Fig. 4).

Table 2
Key information of DENV-2 peptides (protein type, target protein, sequence, length/position, induced T cell type)

Protein Type	Target Protein	Sequence	Peptide Length (Position)	Induced T Cell Type
Structural	prM	AYTIGTTHF	9aa(M 141–149)	CD8+
Structural	E	SYSMCTGKF	9aa(E 298–306)	CD8+
Structural	E	RHVLGRLITVNPIVT	15aa(E 345–359)	-
Structural	E	EPGQLKLNWFKKGSS	15aa(E 383–397)	-
Non-Structurall	NS1	KFQPESPSKL	10aa(NS1 33–42)	CD8+
Non-Structurall	NS1	VFTTNIWLKL	10aa(NS1 162–171)	CD8+
Non-Structurall	NS2A	TYLALLAAF	9aa(NS2A 73–81)	CD8+
Non-Structurall	NS4A	WYAQIQPHWI	10aa(NS4A 96–105)	CD8+
Non-Structurall	NS4B	PYDPKFEKQL	10aa(NS4B 159–168)	CD8+
Non-Structurall	NS4B	SYLAGAGLLF	10aa(NS4B 228–237)	CD8+
Non-Structurall	NS5	WYMWLGARFL	10aa(NS5 475–484)	CD8+
Non-Structurall	NS5	KKLAEAIFKL	10aa(NS5 562–571)	CD8+
Non-Structurall	NS5	TFTNMEAQLI	10aa(NS5 611–620)	CD8+

4. Discussion

DENV−2, recognized as the most pathogenic among the four serotypes, exerts a significant influence on public health containment strategies due to its global epidemiological trends and molecular variation characteristics [25]. This study systematically analyzed the global prevalence, genotype distribution, genomic variations, and their relationship with immune responses of DENV−2 between 2014 and 2024, revealing several key evolutionary features associated with its transmission.

Global surveillance data indicate that DENV−2 infection cases have undergone a consistent increase over the past decade and explosive growth has been particularly observed in Southeast Asia, South America, and East Africa [26]. Notably, autochthonous transmission cases have been reported in European regions such as France [27] and Italy [28], suggesting the gradual spread of DENV−2 into traditional non-endemic areas, likely associated with climate warming and increased international population mobility.

Regarding genotype composition, phylogenetic analysis indicates that the cosmopolitan genotype has become the dominant lineage globally. Its widespread distribution reflects significant advantages in adapting to different hosts and environments. The rapid replacement of other genotypes by the cosmopolitan genotype in Asia, Africa, and South America suggests potential adaptive evolutionary advantages [29, 30]. In contrast, the prevalence of the American genotype has relatively declined in Latin America, while the Asian/American recombinant genotype plays an increasingly important role in transmission across both Asia and the Americas.

Further analysis of the structural and non-structural protein regions of the DENV−2 genome revealed that, although the overall genome is relatively conserved, there is a considerable number of recombination events and non-synonymous mutations in the non-structural protein regions, particularly in the NS1–NS3 and NS5 segments [31]. Notably, the non-synonymous mutation rate in the C-prM-E region of the Sylvatic strain EF105389.1 reached 36.5%, significantly higher than that in human epidemic strains, suggesting potential adaptation for cross-species transmission [32, 33]. Additionally, specific mutations (such as K→N) in domain III of the E protein may affect viral binding efficiency to host receptors, thereby altering viral infectivity [34].

In terms of immune responses, this study identified 13 DENV−2-specific MHC epitopes, 69.2% of which were located in non-structural proteins, with the highest concentration in NS5 and the E protein. These epitopes were predominantly short peptides of 9–10 amino acids, consistent with the characteristics of typical MHC class I-restricted CD8 + T-cell epitopes [35, 36]. Domain III of the E protein and the amino acid region 475–620 in NS5 were identified as key regions possessing both B-cell and T- cell immunogenicity [24], highlighting their significant value for vaccine development.

In summary, this review systematically elucidates the global spread trends, genotype succession patterns, and molecular basis of DENV−2 over the past decade, emphasizing the importance of enhanced global genomic surveillance and cross-regional collaboration. Future vaccine design and therapeutic strategies should focus on conserved and highly immunogenic epitopes within NS5 and the E protein [35, 37], while incorporating considerations of genotype-specific variations for optimization, to effectively counter the evolving threat posed by DENV−2.

Declarations

CRediT authorship contribution statement

Qun Chen: Data curation, Methodology, Software, Visualization, Investigation, Writing – review & editing. **Peipei Ye**: Data curation, Methodology, Software, Visualization, Investigation. **Mengye Ma**: Data curation, Methodology, Software, Visualization, Investigation. **Zhu Chen**: Data curation, Methodology, Software, Visualization, Investigation. **Liming Jiang**: Supervision.

Declaration of conflict of interest

The authors declare no competing financial interest.

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References

1. Seixas BA, Giovanni Luz J, Pinto Junior K V (2024) Atualização Clínica sobre Diagnóstico, Tratamento e Prevenção da Dengue [Clinical Update on Diagnosis, Treatment and Prevention of Dengue]. Acta Med Port 37(2):126–135. https://doi.org/10.20344/amp.20569
2. Naddaf M (2023) Dengue is spreading in Europe: how worried should we be? Nature, 10.1038/d41586-023-03407–6. Advance online publication https://doi.org/10.1038/d41586-023-03407–6
3. Vazeille M, Rosen L, Mousson L, Failloux AB (2003) Low oral receptivity for dengue type 2 viruses of Aedes albopictus from Southeast Asia compared with that of Aedes aegypti. Am J Trop Med Hyg 68(2):203–208
4. World Health Organization (2024) Afghanistan infectious disease outbreak situation reports[EB/OL].https://www.emro.who.int/afg/information-resources/infectious-disease outbreak-situation-reports.html

5. Malavige GN, Sjö P, Singh K, Piedagnel JM, Mowbray C, Estani S, Lim SCL, Siquierra AM, Ogg GS, Fraisse L, Ribeiro I (2023) Facing the escalating burden of dengue: Challenges and perspectives. PLOS global public health 3(12):e0002598. https://doi.org/10.1371/journal.pgph.0002598
6. Haider N, Asaduzzaman M, Hasan MN, Rahman M, Sharif AR, Ashrafı SAA, Lee SS, Zumla A (2023) Bangladesh's 2023 Dengue outbreak - age/gender-related disparity in morbidity and mortality and geographic variability of epidemic burdens. Int J Infect diseases: IJID : official publication Int Soc Infect Dis 136:1–4. https://doi.org/10.1016/j.ijid.2023.08.026
7. Dash PK, Sharma S, Soni M, Agarwal A, Sahni AK, Parida M (2015) Complete genome sequencing and evolutionary phylogeography analysis of Indian isolates of Dengue virus type 1. Virus Res 195:124–134. https://doi.org/10.1016/j.virusres.2014.08.018
8. Perera R, Kuhn RJ (2008) Structural proteomics of dengue virus. Curr Opin Microbiol 11(4):369–377. https://doi.org/10.1016/j.mib.2008.06.004
9. Gebhard LG, Filomatori CV, Gamarnik AV (2011) Functional RNA elements in the dengue virus genome. Viruses 3(9):1739–1756. https://doi.org/10.3390/v3091739
10. Iglesias NG, Gamarnik AV (2011) Dynamic RNA structures in the dengue virus genome. RNA Biol 8(2):249–257. https://doi.org/10.4161/rna.8.2.14992
11. Weaver SC, Vasilakis N (2009) Molecular evolution of dengue viruses: contributions of phylogenetics to understanding the history and epidemiology of the preeminent arboviral disease. Infection, genetics and evolution. J Mol Epidemiol evolutionary Genet Infect Dis 9(4):523–540. https://doi.org/10.1016/j.meegid.2009.02.003
12. Diamond MS, Pierson TC (2015) Molecular Insight into Dengue Virus Pathogenesis and Its Implications for Disease Control. Cell 162(3):488–492. https://doi.org/10.1016/j.cell.2015.07.005
13. Bosch I, Reddy A, de Puig H et al (2020) Serotype-specific detection of dengue viruses in a nonstructural protein 1-based enzyme-linked immunosorbent assay validated with a multi-national cohort. PLoS Negl Trop Dis 14(6):e0008203. https://doi.org/10.1371/journal.pntd.0008203
14. Kok BH, Lim HT, Lim CP, Lai NS, Leow CY, Leow CH (2023) Dengue virus infection - a review of pathogenesis, vaccines, diagnosis and therapy. Virus Res 324:199018. https://doi.org/10.1016/j.virusres.2022.199018
15. Soo KM, Khalid B, Ching SM, Chee HY (2016) Meta-Analysis of Dengue Severity during Infection by Different Dengue Virus Serotypes in Primary and Secondary Infections. PLoS ONE 11(5):e0154760. https://doi.org/10.1371/journal.pone.0154760
16. Sirisena N, Mahilkar PDN, Sharma S, Jain C, J., Sunil S (2021) Concurrent dengue infections: Epidemiology & clinical implications. Indian J Med Res 154(5):669–679. https://doi.org/10.4103/ijmr.IJMR_1219_18
17. Messina JP, Brady OJ, Scott TW, Zou C, Pigott DM, Duda KA, Bhatt S, Katzelnick L, Howes RE, Battle KE, Simmons CP, Hay SI (2014) Global spread of dengue virus types: mapping the 70 year history. Trends Microbiol 22(3):138–146. https://doi.org/10.1016/j.tim.2013.12.011
18. Tissera HA, Jayamanne BDW, Raut R, Janaki SMD, Tozan Y, Samaraweera PC, Liyanage P, Ghouse A, Rodrigo C, de Silva AM, Fernando SD, Severe Dengue Epidemic, Lanka S (2020) 2017. Emerging infectious diseases, 26(4), 682–691. https://doi.org/10.3201/eid2604.190435
19. Aragão CF, Pinheiro VCS, Neto N, Silva JP, Pereira EVPD, Nascimento GJG, Castro BLSD, Maia KDS, Catete AM, Martins CP, Tadei LC, Silva WP, S. P. D., Cruz ACR (2019) Natural Infection of Aedes aegypti by Chikungunya and Dengue type 2 Virus in a Transition Area of North-Northeast Brazil. Viruses 11(12):1126. https://doi.org/10.3390/v11121126
20. Guzman MG, Harris E, London (2015) England), 385(9966), 453–465. https://doi.org/10.1016/S0140–6736(14)60572–9
21. Buchan DWA, Jones DT (2019) The PSIPRED Protein Analysis Workbench: 20 years on. Nucleic Acids Res 47:W402–W407. https://doi.org/10.1093/nar/gkz297
22. Duvaud S, Gabella C, Lisacek F, Stockinger H, Ioannidis V, Durinx C (2021) Expasy, the Swiss Bioinformatics Resource Portal, as designed by its users. Nucleic Acids Res 49(W1):W216–W227. https://doi.org/10.1093/nar/gkab225
23. Mishra B, Balaji A, Beesetti H, Swaminathan S, Aduri R (2020) The RNA secondary structural variation in the cyclization elements of the dengue genome and the possible implications in pathogenicity. Virusdisease 31(3):299–307. https://doi.org/10.1007/s13337-020-00615-w
24. Duan ZL, Liu HF, Huang X, Wang SN, Yang JL, Chen XY, Li DZ, Zhong XZ, Chen BK, Wen JS (2015) Identification of conserved and HLA-A*2402-restricted epitopes in Dengue virus serotype 2. Virus Res 196:5–12. https://doi.org/10.1016/j.virusres.2014.10.022
25. Ni H, Cai X, Ren J, Dai T, Zhou J, Lin J, Wang L, Wang L, Pei S, Yao Y, Xu T, Xiao L, Liu Q, Liu X, Guo P (2024) Epidemiological characteristics and transmission dynamics of dengue fever in China. Nat Commun 15(1):8060. https://doi.org/10.1038/s41467-024-52460-w
26. Begum R, Paul SK, Aung MS, Haque N, Ahmed S, Islam A, Nila SS, Roy S, Jahan A, Sathi FA, Mamun A, Biswas A, J. P., Kobayashi N (2024) Predominance of Dengue virus type 2-genotype II (Cosmopolitan) in Bangladesh, 2023: Presumptive sudden replacement of a prevailing virus strain. New microbes new infections 60–61. https://doi.org/10.1016/j.nmni.2024.101431
27. Cochet A, Calba C, Jourdain F, Grard G, Durand GA, Guinard A, Investigation team, Noël H, Paty MC, Franke F (2022) Autochthonous dengue in mainland France, 2022: geographical extension and incidence increase. Euro surveillance: bulletin Europeen sur les maladies transmissibles = European. Commun disease Bull 27(44):2200818. https://doi.org/10.2807/1560–7917.ES.2022.27.44.2200818

28. Salvo PF, Baldin G, Raffaelli F, Ciccullo A, Borghetti A, Tamburrini E, Ricci R, Di Donato M, Di Giambenedetto S, Torti C (2024) Autochthonous dengue outbreak in Rome, Italy, in 2023. J Travel Med 31(7):taae111. https://doi.org/10.1093/jtm/taae111
29. Gräf T, Ferreira CDN, de Lima GB et al (2023) Multiple introductions and country-wide spread of DENV–2 genotype II (Cosmopolitan) in Brazil. Virus Evol 9(2):vead059. https://doi.org/10.1093/ve/vead059
30. Yenamandra SP, Koo C, Chiang S, Lim HSJ, Yeo ZY, Ng LC, Hapuarachchi HC (2021) Evolution, heterogeneity and global dispersal of cosmopolitan genotype of Dengue virus type 2. Sci Rep 11(1):13496. https://doi.org/10.1038/s41598-021-92783-y
31. Stica CJ, Barrero RA, Murray RZ, Devine GJ, Phillips MJ, Frentiu FD (2022) Global Evolutionary History and Dynamics of Dengue Viruses Inferred from Whole Genome Sequences. Viruses 14(4):703. https://doi.org/10.3390/v14040703
32. Vasilakis N, Holmes EC, Fokam EB, Faye O, Diallo M, Sall AA, Weaver SC (2007) Evolutionary processes among sylvatic dengue type 2 viruses. J Virol 81(17):9591–9595. https://doi.org/10.1128/JVI.02776–06
33. Dieng I, Diallo A, Ndiaye M, Mhamadi M, Diagne MM, Sankhe S, Ndione MHD, Gaye A, Sagne SN, Heraud JM, Sall AA, Fall G, Loucoubar C, Faye O, Faye O (2022) Full genome analysis of circulating DENV–2 in Senegal reveals a regional diversification into separate clades. J Med Virol 94(11):5593–5600. https://doi.org/10.1002/jmv.28027
34. Gromowski GD, Barrett ND, Barrett AD (2008) Characterization of dengue virus complex-specific neutralizing epitopes on envelope protein domain III of dengue 2 virus. J Virol 82(17):8828–8837. https://doi.org/10.1128/JVI.00606–08
35. Weiskopf D, Angelo MA, de Azeredo EL, Sidney J, Greenbaum JA, Fernando AN, Broadwater A, Kolla RV, De Silva AD, de Silva AM, Mattia KA, Doranz BJ, Grey HM, Shresta S, Peters B, Sette A (2013) Comprehensive analysis of dengue virus-specific responses supports an HLA-linked protective role for CD8 + T cells. Proceedings of the National Academy of Sciences of the United States of America, 110(22), E2046–E2053. https://doi.org/10.1073/pnas.1305227110
36. Shi J, Sun J, Wu M, Hu N, Li J, Li Y, Wang H, Hu Y (2015) Inferring Protective CD8 + T-Cell Epitopes for NS5 Protein of Four Serotypes of Dengue Virus Chinese Isolates Based on HLA-A, -B and -C Allelic Distribution: Implications for Epitope-Based Universal Vaccine Design. PLoS ONE 10(9):e0138729. https://doi.org/10.1371/journal.pone.0138729
37. Goh JZH, De Hayr L, Khromykh AA, Slonchak A (2024) The Flavivirus Non-Structural Protein 5 (NS5): Structure, Functions, and Targeting for Development of Vaccines and Therapeutics. Vaccines 12(8):865. https://doi.org/10.3390/vaccines12080865

Figures

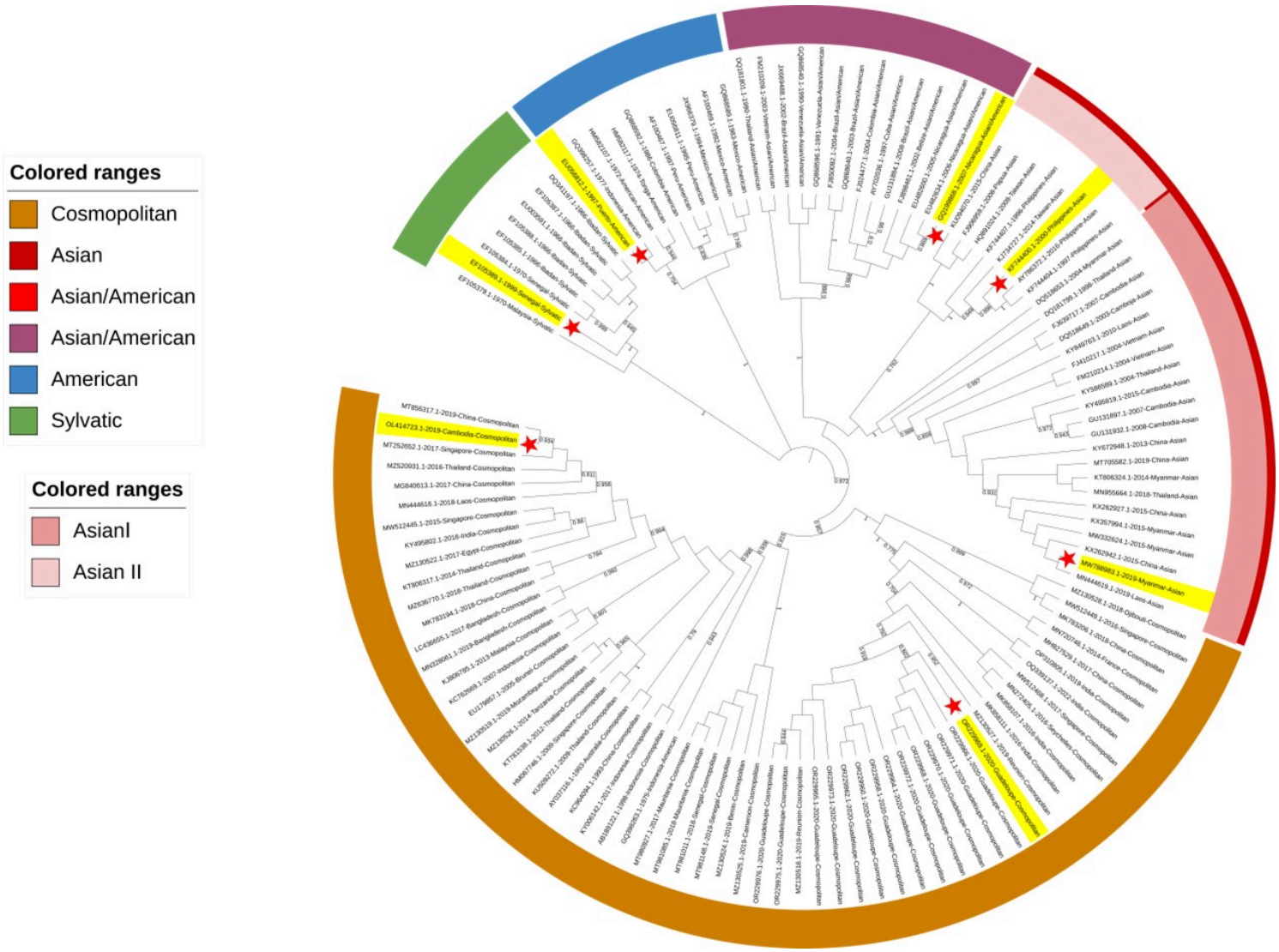


Figure 1

Phylogenetic clustering analysis of representative DENV-2 strains (color-coded by genotype, with strain information including accession number, sampling year, region, and genotype). Phylogenetic Analysis, based on the complete sequence, was conducted using MEGA 7.0 software (maximum likelihood method) with 1,000 Bootstrap Replicates. Different genotype representative strains are marked with yellow.

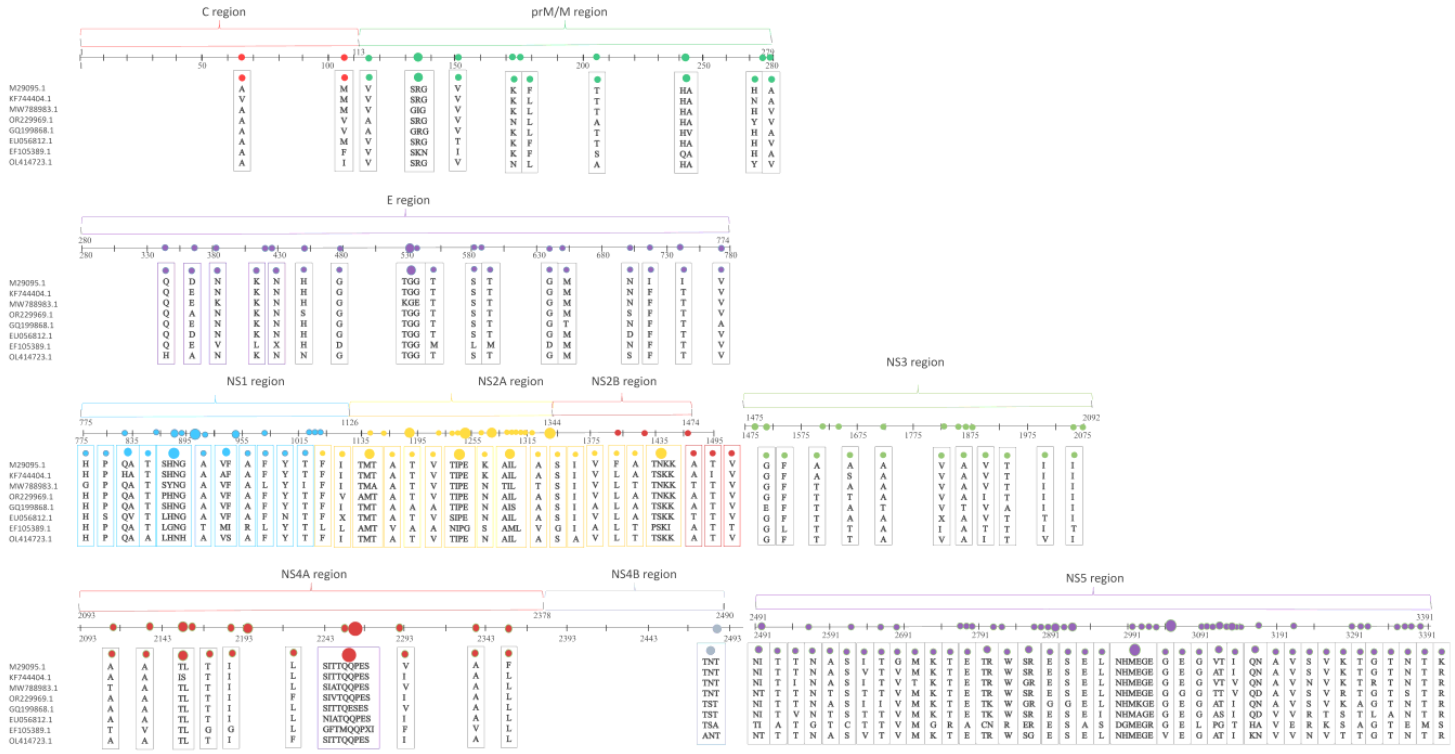


Figure 2

Amino acid substitutions in KF744404.1, MW788983.1, OR229969.1, GQ199868.1, EU056812.1, EF105389.1, OL414723.1compared to DEN-2SS M29095.1, with detailed regional distribution (C-prM/M-E, NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5 regions) and amino acid position labeling. The labeled numbers represent the amino acid position.

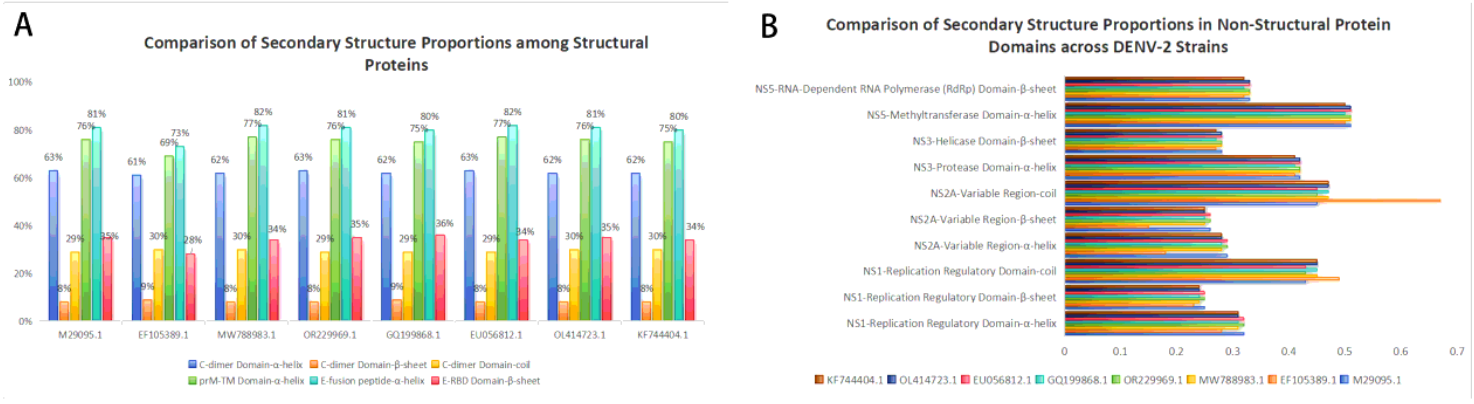


Figure 3

Panels A and B depict the secondary structure composition of functional domains in structural (A) and non-structural (B) proteins across DENV-2 strains. Structural proteins show high conservation, while non-structural proteins exhibit strain-specific structural divergence, reflecting evolutionary specialization in viral protein architecture.

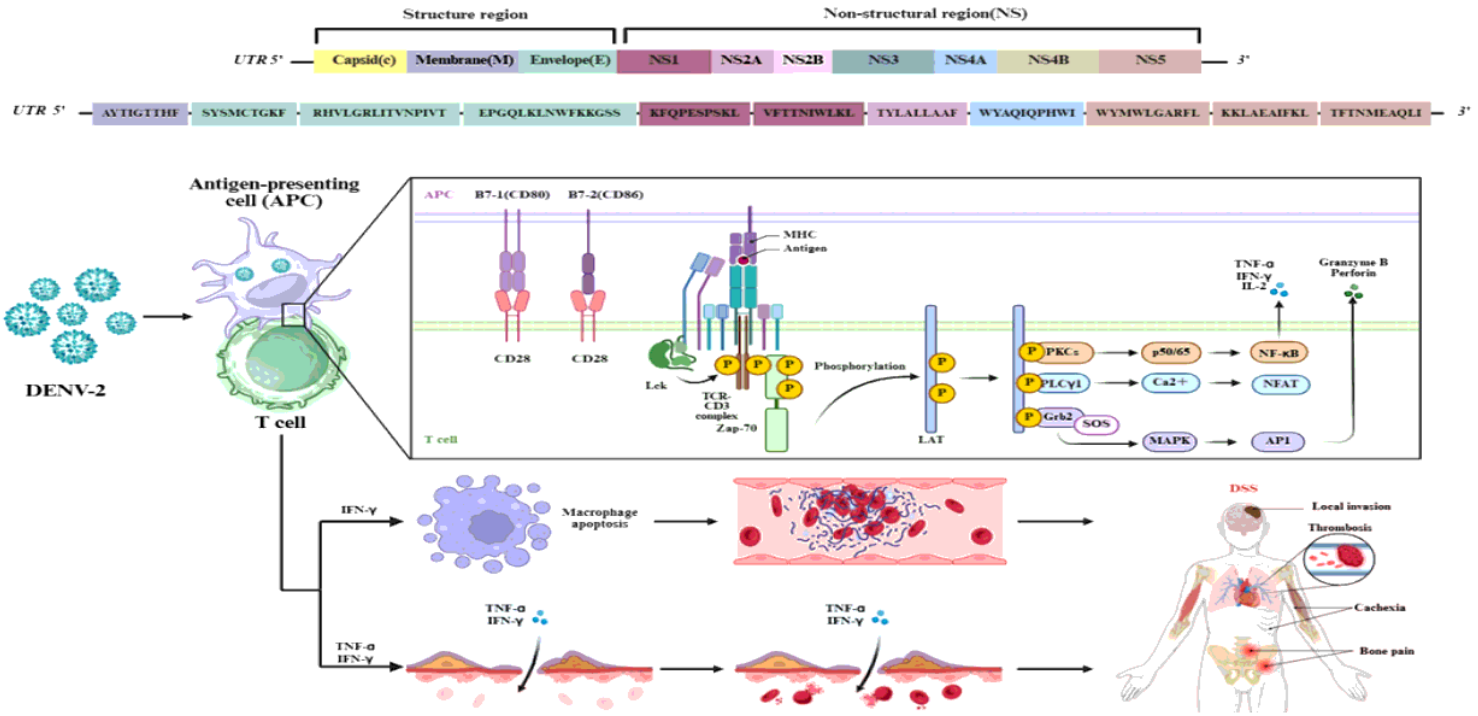


Figure 4

DENV-2 genome structure, CD8+ T cell signaling pathway activation, and the association with antibody-dependent enhancement (ADE).