Molecular characterization of the viral structural gene of the first dengue virus type 2 outbreak in Hunan Province, inland China

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Research article

Keywords: Dengue virus, structural protein, phylogenetic trees, protein structure, selection pressure

Posted Date: January 15th, 2020

DOI: https://doi.org/10.21203/rs.2.20904/v1

Version of Record: A version of this preprint was published on February 10th, 2021. See the published version at https://doi.org/10.1186/s12879-021-05823-3.

Abstract

Background: An unexpected dengue outbreak occurred in the Hunan Province in 2018. This is the first dengue outbreak in this area of inland China resulting 172 infected.

Methods: To verify the causative agent of this outbreak and investigate gene characterization, the structural protein C/prM/E genes of viruses isolated from local residents were sequenced followed by mutation, phylogenetic analysis. The recombination, selection pressure, potential secondary structure and three-dimensional structure analysis were also performed.

Results: Phylogenetic analysis revealed that all epidemic strains were classified as the cosmopolitan DENV-2 genotype, closest to the Zhejiang strain (MH010629, 2017) and then Malaysia strain (KJ806803, 2013). Compared with the DENV-2SS, 151 base substitutions were found in 89 sequences of isolates, resulting in 20 nonsynonymous mutations, of which 17 mutations existed among all samples (two in capsid protein, six in prM/M, and nine in envelope proteins). Moreover, amino acid substitutions at 602 th (E322:Q→H) and 670 th (E390: N→S) may result in heightened virulence of the epidemic strains. One new DNA-binding site and five new protein binding sites were observed. Two polynucleotide-binding sites and seven protein binding sites were lost compared with DENV-2SS. Meanwhile, five changes were found in helix regions. The helical transmembrane and disordered regions have minor changes. Protein tertiary structure prediction revealed the 429 th amino acid of E proteins was switch from histamine (positively charged) to asparagines (neutral) in 89 isolate strains. No recombination events or positive selection pressure sites were detected. To our knowledge, this study is the first gene analysis of epidemic strain in the first dengue outbreak in Hunan Province, inland China.

Conclusions: The causative agent is likely to come from Zhejiang Province, a neighbouring Province where dengue fever broke out in 2017. This study may help understand the intrinsic geographical relatedness of DENV-2 and contributes further to research on pathogenicity and vaccine development.

Background

Dengue fever (DF) is an ancient disease with a history of approximately 200 years, which is caused by four different but closely related dengue viruses (DENV-1, DENV-2, DENV-3, and DENV-4) and is mainly transmitted by female aedes aegypti or female aedes albopictus [1, 2]. It occurs in tropical and subtropical urban and semi-urban areas around the world. The global dengue epidemic has spread in recent decades, from nine endemic countries before 1970 to 128 in 2012 [3]. The incidence of dengue has also increased dramatically, from 1.2 million in 2008 to 3.9 million in 2015 [4, 5]. More than 2.38 million cases in the Americas region alone during 2016, Brazil accounted for about 1.5 million cases, and more than 375,000 cases in the western Pacific region, including 176,000 cases in the Philippines, and 100,000 cases in Malaysia [6]. However, the number of dengue cases reported in the Americas was 580,000 in 2017, approximately 78.9% fewer than last year. According to the data provided by the World Health Organization (WHO), the number of cases in the first quarter of 2018 decreased by 27% compared with

the same period of 2017, mainly occurring in countries such as Paraguay, Argentina, Bangladesh, Cambodia, India, Myanmar, Malaysia, Pakistan, Thailand, Yemen, and China, and mainly caused by DENV-1 and DENV-2 serotypes.

DF has become a serious public health problem in China, according to data provided by the Chinese Center for Disease Control (CCDC), with 757,243 people infected in the past 42 years [7, 8], largely occurring in Hainan [9], Guangdong [10, 11], Zhejiang [12, 13], Fujian [14, Taiwan, and Yunnan [15–18]. In 2018, an unexpected and first-time dengue outbreak occurred in Hunan, an inland province of China. The first dengue fever case was reported on September 2. Till October 6, 172 infected individuals were confirmed as NS1-positive, with one death. 73 cases were confirmed during September 8 to September 14, accounting for 76.04%. The ratio of female and male infected patients was 1.04 to 1 (49:47), with an average age of 49.5 (ranging from 11 to 84 years old). It should be noted that no dengue case was found in this area from 2000 to 2013, and there were only five imported cases during 2014–2017 with no local cases.

This was the first dengue outbreak in Hunan, interior province of China. It provided us an early warning that the dengue fever has been gradually spread inland from China's coastal and border regions and highlighted the urgent need to monitor the cross-border and cross-regional spread of dengue virus. The purpose of this paper was to verify the causative agent and analyze the molecular characteristics of the epidemic strain in this outbreak.

Methods

The geographic analysis of Hunan province and study design

The geographical distribution map of dengue fever in China over the years was made using Chinese mapping and drawing software. Blood samples of patients were collected from two local hospitals responsible for the treatment of DENV patients (Qiyang People's Hospital and the Nongshan Hospital) during the 2018 dengue outbreak. The dengue fever epidemic situation in the surrounding areas of Hunan province was also analyzed.

Dengue virus RNA extraction and identification

Viral RNA were extracted from 140 μl of serum using the QIAamp viral RNA mini kit (Qiagen, Hilden, Germany; No.52906) and then reverse transcribed into cDNA using the Prime Script TM II 1st Strand cDNA Synthesis Kit (Takara Bio, Shiga, Japan; No.6210A). Universal primer of dengue virus and the specific primers of the four serotypes (Table S1) were used for polymerase chain reaction (PCR), and the type was identified. The reaction conditions in each 25-μl volume were denaturation at 95℃ for 5 min, followed by 30 cycles of denaturation at 95℃ for 30 s, annealing at 55℃ for 30 s, and elongation at

72℃ for 30 s, with a final elongation step at 72℃ for 7 min. The PCR products were then subjected to gel electrophoresis.

Primer design

A total of three synthetic oligonucleotide primer pairs F1/R1, F2/R2, and F3/R3 (Table S2) were designed to amplify overlapping fragments with sizes of 2,325 nucleotides spanning the entire structural protein genome of DENV-2. All primers were designed using SnapGene software (version 3.2.1), based on the Japan strain (GenBank accession no. M29095). All primers were synthesized and purified by Sangon Biotech Co., Ltd. (Shanghai, China).

Gene amplification and sequencing

PCR was performed with the following protocol (50-μl volume): denaturation at 95℃ for 3 min, followed by 30 cycles of denaturation at 95℃ for 10 s, annealing at 60℃ for 90 s, and elongation at 72℃ for 30 s, with a final elongation step at 72℃ for 7 min. The PCR products were confirmed by agarose gel electrophoresis and sequenced at Sangon Biotech Co., Ltd. (Shanghai, China). Both forward and reverse sequencing were done.

Sequence and phylogenetic analysis

Sequence of the structural protein genes (C/prM/E) were aligned by MEGA 7.0 and compared with 133 dengue virus (DENV) reference strains, including four serotypes of standard strains (Table S3), which were collected from websites (https://www.viprbrc.org). Phylogenetic analysis was performed using MEGA 7.0 through the ML phylogeny test with a bootstrap of 1,000 replications.

Molecular characteristics analysis

A total of 89 nucleotide sequences were assembled using BioEdit 7.1.3 (http://www.mbio.ncsu.edu/bioedit/bioedit.html) then uploaded to Genbank through Sequin Application (version 15.50), and BankIt was used to access the National Center for Biotechnology Information (NCBI) GenBank database (https://www.ncbi.nlm.nih.gov/genbank/). Next, the nucleotide sequence and translated amino acid sequence mutations of the structural proteins of these 89 strains were analyzed with BioEdit and Molecular Evolutionary Genetics Analysis (MEGA) software version 7.0. The secondary structure of structural proteins of DENV-2 epidemic and reference strains were predicted with the Predict Protein server (https://www.predictprotein.org/).

Recombination and selection pressure analysis

The Detection of Recombination Using a Genetic Algorithm (GARD) [19] server of Datamonkey [20] in the online software was used for automatic analysis of reorganization events of the structural protein regions with 775 codons of 130 DENV-2 reference sequences in Table S4 and 89 epidemic strains in our study. The phylogenies server in the software was used for analysis of selection pressure. In this research, four methods, the Fixed Effect Likelihood (FEL) [21], Internal Fixed Effect Likelihood (IFEL) [22], Mixed Effect Evolution Model (MEME) [23], and Rapid Unbiased Bayesian Approximation (FUBAR) [24], were adopted to estimate the locus specific selection pressure. At least three of the four methods meet the requirement of $\omega > 1 (\omega = \beta/\alpha)$, and the p-value < 0.1 or Posterior Prob ($\alpha < \beta$) >0.9. The positive selection of this site can be inferred.

Results

The Geographic Analysis of Hunan Province and Study Design

The geographic relationships between Hunan and the DENV outbreak areas in China were analyzed first. The results showed that Hunan had become a central area of the DENV epidemic, which was surrounded by Yunnan, Guangdong, Guangxi, Hainan, Fujian, Zhejiang and the other dengue outbreak areas (Fig. 1) (The map in the figure was drawn by ourselves. Part of the data in the map quoted from Zhao [25], and part was provided by Centers for Disease Control and Prevention of Hunan Province).

During the DENV outbreak in Qiyang County, Hunan from September 2018, a total of 260 serum samples from fever patients were collected, and all of these cases were confirmed to be NS1-positive through colloidal gold testing. Of these, 96 DENV-positive serum samples were screened out from patients whose fever courses were shorter than 5 days. Seven strains were amplified in C6/36 cells for over 6 days to construct a viral seed library of Hunan DENV. Eighty-nine viral RNAs were successfully extracted directly from these serum samples, followed by gene sequencing of the DENV structural protein C/prM/E genes. The phylogenetic analysis, recombination and selection pressure analysis, potential secondary structure prediction based on structural gene sequences originating from epidemic strains were performed to understand the genetic characterization, potential source, and evolution. The study design and the following disposition of study subjects are shown in Fig. 2.

Phylogenetic analysis

The C/prM/E gene sequences of 89 strains were uploaded to Genbank (http://www.info@ncbi.nlm.nih.gov/GenBank/index.html) (GenBank ID: MK543451-MK543470, MK543472-MK543478, MK543480-MK543492, MK949396-MK949438) through Sequin Application (version15.50). One hundred twenty-three different representative DENV-2 strains and four serotypes of

standard strains of E protein of dengue virus (DENV) were selected to construct phylogenetic trees with MEGA software version 7.0. The result exhibited that all 89 strains in this study were cosmopolitan DENV-2 genotypes. The closest relative was the Zhejiang strain (MH010629, 2017), followed by strains from Malaysia (KJ806803, 2013), Bali (KT806318, 2014), Indonesia (KT781561, 2014), and the Philippines (KU517847, 2015) (Fig. 3).

Bases and amino acid mutations

Through amplification, three structural protein-overlapped fragments of 89 epidemic strains were obtained. After sequencing, the proteins were effectively spliced, and the length of coding nucleotide sequences was 2,325 nt, 775 amino acids were encoded, the homology between isolates was 99.7-100%, and the amino acid (AA) sequence of E protein was highly conserved. By comparison, the comparability of nucleotide and amino acid sequences between the 89 epidemic strains and DENV-2SS were 93.5 and 97.8%, respectively. Two hundred fifteen bases had mutations in the structural protein region of epidemic strains, among which 195 were synonymous mutations and 20 were nonsynonymous mutations, leading to 17 AA substitutions (Fig. 4). Two AA substitutions at 104^{th} (C104: M→I) and 108^{th} C108: L→M) were observed in protein C in isolate strains, six amino acid mutations including 143^{th} (M29: D→N), 166^{th} (M52: K→N), 196^{th} (M82: T→A), 241^{th} (M127: I→V, 262^{th} (M148: H→Y), and 266^{th} (M152: A→V) occurred in the structural protein prM/M, and nine amino acid mutations including 332^{th} (E52: Q→H), 351^{th} (E:71 D→A), 406^{th} (E126: K→E), 409^{th} (E129: V→I), 429^{th} (E149: H→N), 444^{th} (164: I→V), 602^{th} (E322: I→V), 670^{th} (E390: N→S), 742^{th} (E462: I→V) were found in structural protein E (Fig. 4). Only one amino acid mutation (I431V/A) was observed in all 89 epidemic strains compared with the nearest related strain from Zhejiang (MH010629, 2017).

Potential secondary structure of the structural protein region

The protein secondary structure among DENV-2 standard strain KM204118 and three randomly selected sequences (HNQY2018014, 021, and 028) from the 89 isolate strains were predicted. Compared with DENV-SS, Hunan epidemic strains had missed one nucleotide-binding site (site 6) and one DNA-binding site (site 18), as well as one protein binding region (sites: 4 and 5) in the capsid protein (Fig. S1), while one new DNA-binding site (site 74) and two new protein binding sites (19 and 29) were observed in isolate strains. Moreover, variations were found in the disordered region among Hunan epidemic strains, DENV-2SS and Zhejiang/2017 (Fig. S1). In the prM/M region, which contained 166 amino acids, the protein secondary structure of the epidemic strains was highly consistent with that of the Zhejiang strain (Fig. S2). However, compared to the DENV-2SS, three protein binding regions disappeared in Hunan epidemic strains (sites:122, 133, and 220), and one novel protein binding region emerged (site 144). Additionally, one helical transmembrane region of the isolates visibly differed from the DENV-2SS, and

eight significant changes were observed in the buried and exposed region, while no noticeable variation was found in the strand and helix region (Fig. S2). Three protein binding sites (sites: 584, 596, and 642) disappeared at the 495AA locus of E protein, one protein binding location (site 377) was updated in Hunan isolates, four considerable alterations were observed in exposed and buried regions, and minor changes were found in the helical transmembrane and disordered region (Fig. 5). Meanwhile, there were 22 changes in strand regions. Of them, 11 were new (120, 166, 192, 309, 334, 347, 446, 455, 512, 582-584, 591), 11 were missing (101, 102, 124, 141, 207, 290, 294, 553, 607, 636, 651, 692-695), and nearly 70% of changes occurred in E proteins. Nevertheless, compared with the Zhejiang 2017 strain, there was no significant change in protein binding region and polynucleotide-binding region in structural protein (C, prM/M, and E) (Fig. 5, Fig. S1, and Fig. S2).

Possible three-dimensional structure of the structural protein E genes

The possible three-dimensional structure of structural proteins of the representative epidemic strains (HNQY2018014, 021, and 028) were predicted and compared with DENV2-SS and Zhejiang/2017 strain. Homology modeling revealed that five strains had the same three-dimensional structure. In addition, binding sites were also predicted by the 3DLigandSite ligand binding site prediction server, four protein binding sites were observed in DENV-2SS (HIS429, ALA430, THR435, and GLY436) (Fig. 6E). Hunan epidemic strains and the Zhejiang/2017 strain have the same binding sites at ASN429, THR435, and GLY436) (Fig. 6D). HNQY2018028 has two different binding sites (429 and 430) compared to DENV-2SS (Fig. 6) and one diverse binding site (429) compared to Zhejiang/2017.

Recombination and election pressure analysis

RDP4 software was used to analyze potential recombination events among HNQY2018001-HNQY2018089 and other representative DENV-2 virus strains. Preliminary analysis results showed that no recombination event may occur in these DENV-2 strains ($p<0.05$). The structural proteins of 202 strains were analyzed, including 113 representative strains of DENV-2 and 89 isolate strains. The results showed that the MEME method identifies the maximum number of actively selected sites (n = 16). However, the FEL, IFEL and FUBAR methods indicated that all 775 sites were under negative pressure (Table 1). Therefore, no significant evidence of positive selection was presented in at least three different methods, so positive sites of selection pressure at these sites cannot be determined.

Discussion

In China, dengue fever mainly occurs in Guangdong, Hainan, Zhejiang, Fujian, Taiwan, Guangxi, and other coastal regions, or in Yunnan Province and its borders with South Asian countries. Only scattered cases have been reported in inland China, but no large-scale dengue epidemic has been reported in the inland

area to date. Hunan is an inland province of China, located near 30 degrees north latitude, with a warm and humid climate from June to November, providing a natural environment for aedes albopictus breeding. Hunan province is located near Guangdong, Guangxi, Zhejiang, and other areas with high incidence of dengue fever. The total number of dengue infections in China in 2018 was 5,106, including 3,250 in Guangdong province, 217 sporadic cases in Zhejiang, and 172 cases in Hunan. This was the first dengue outbreak in Hunan and China's interior province, and it provided us an early warning that the dengue fever has gradually spread inland from China's coastal and border regions and highlighted the urgent need to monitor the cross-border and cross-regional spread of dengue virus.

In this study, we collected serum from 260 patients with fever in Qiyang county, Hunan province, and confirmed 96 cases with positive NS1. Seven of the cases were treated with virus amplification culture on C6/36 cells to preserve the seeds, and another 89 viral RNAs were extracted and structural protein genomes (HNQY2018001-089) were obtained by amplification of overlapping fragments with a length of 2,325 nucleotides. Phylogenetic tree analysis showed that all isolate strains were cosmopolitan DENV-2 genotypes, and all located on one cluster of the ML tree and were closely related to the Zhejiang strain (2017, MH110588). Additionally, it is also closely related to the following four strains: Malaysia (KJ806803, 2013), Bali (KT806318, 2014), Indonesia (KT781561, 2014) and the Philippines (KU517847, 2015). This result suggested that the DENV-2 epidemic in Hunan possibly imported from Southeast Asian countries, such as Malaysia, Indonesia or the Philippines, passed through Zhejiang first, and then further spread to Hunan or directly from these neighboring countries. Compared with the structural protein C/prM/E of standard strains, 17 amino acid substitutions occurred in all 89 epidemic strains. prM-E protein is the main structural protein of flavivirus, which is related to virulence, host affinity, virus adsorption, penetration, and cell fusion [26]. Hydrophobic amino acids play an important role in maintaining the tertiary structure of proteins due to their hydrophobic interactions and may impact the virulence of the virus. Tamm et al. found that hydrophobic domains affect the virulence potential of the YadA protein of Yersinia enterocolitica [27]. Sainz et al. determined that single hydrophobic amino acids play an important role in transcriptional activation in vivo [28]. In our study, three hydrophobic amino acids in CDS region mutated into hydrophilic ones at 196th (M82: T→A), 262th (M148: H→Y), and 351th (E71: D→A). In addition, neutral amino acids became basic amino acids at 332th (E52: Q→H), and two positive electricity amino acids converted into negative ones at sites 406th (E126: K→E) and 429th (E139: H→N), mutations in these amino acids have not been reported, and changes in polarity or charge of amino acids may affect the functions of prM and E proteins, but these speculations need further study to confirm. Moreover, Moreland et al. defined that this region of amino acids (E295 ~ E395) of dengue virus E protein domain III, which is the immunoglobulin G (IgG) immunoglobulin-like folding and plays an important role in mediating the fusion of virus and host receptor [29]. In this study, there were two site changes in the EDIII domain 602th (E322: I→V) and 670th (E390: N→S), it has been proved that the mutation of E390 from N amino acid to S amino acid can enhance the replication ability of virus [30], but the influence of E322 amino acid mutation remains to be further confirmed.

The change of protein secondary structure will affect the enzyme activity. Compared with DENV-2 standard strain (KM204118), eight protein binding sites (4, 5, 122, 120, 133, 584, 596, and 642) and two

polynucleotide-binding sites (6 and 18) were missed. Simultaneously, four new protein binding sites (19, 29, 144, and 377) and one polynucleotide-binding site (74) emerged. Furthermore, approximately eight obvious changes were taken place in buried area and exposed region. All of the above changes may lead to the diversification of protein structure domain, further influencing the protein function. Homologous modeling and prediction of the possible 3D structure of structural proteins showed that epidemic strains and DENV2-SS had the similar 3D structure and 4 predicted protein binding sites, while, it is notable that the 429th binding site was different among them (DENV2-SS: His 429; Zhejiang/2017 and Hunan epidemic strain: ASN 429).

The analysis showed that there was no recombination event among the Hunan epidemic strains and 130 DENV-2 reference sequences, and no distinct positive selection site in structural protein, which contained 775 amino acids, suggesting that these structural protein coding genes were conservative.

Conclusions

This study reported the characteristics of the structural protein genome in DENV-2 originating from the 2018 outbreak in Hunan, inland China. This will benefit the research of 2018 and later follow-up studies of DENV outbreak in China and Southeast Asia. Our finding also showed that the transmission region of DENV has gradually spread from China's border and coastal areas to inland China. It provided us a warning that the dengue fever epidemic in China has become increasingly serious and difficult to control and emphasized the urgent need to monitor the cross-border spread of DENV.

Abbreviations

AA: Amino acid; CCDC: Chinese center for disease; DENV: Dengue virus; DENV-1/2/3/4: Dengue virus serotype 1/2/3/4; DF: Dengue fever; DENV-2SS: Dengue virus serotype 2 standard strain; FEL: Fixed effect likelihood; FUBAR: Rapid unbiased Bayesian approximation; GARD: Detection of recombination using a genetic algorithm; IFEL: Internal fixed effect likelihood; IgG: Immunoglobulin G; MEME: Mixed effect evolution model; MEGA: Molecular evolutionary genetics analysis; NCBI: National center for biotechnology information; NS1: Non-structural protein 1; NT: Nucleotides; PCR: Polymerase chain reaction; WHO: World health organization.

Declarations

Ethics approval and consent to participate

Written informed consent was obtained from all participants and each one was informed of the purpose of the study, and written informed consent for participation in the study was obtained where participants are children (under 16 years old) from their parent or guardian.The study protocol was approved by the Institutional Ethics Committee (Institute of Medical Biology, Chinese Academy of Medical Sciences, and Peking Union Medical College).

Consent for publication

Not Applicable.

Availability of data and material

The datasets generated and analyzed during the present study are available from the corresponding author on reasonable request.

Competing interests

The authors report no conflicts of interest in this work.

Funding

This research was supported by the Foundation of the CAMS (Chinese Academy of Medical Sciences) Initiative for Innovative Medicine (CAMS-I2M) (2017-I2M-2-006), the National Natural Science Foundation of China (31970868), Major Projects and Key Research and Development Plans of Yunnan Province (2019ZF004), the Natural Science Foundation of Yunnan Province (2016FA029), Yunnan Medical Discipline Leader Project (D-201605).

Authors' contributions

QS, and SF contributed to the design of the study. JG was responsible for data collection, analysis and manuscript writing. MQ, and ZH provided software technology support. XD, and XG provided samples collection support. SD, JC, YP, JC and YY provided experiment technology support. All the authors agree to the final version of the submission.

Acknowledgements

The authors express their heartfelt thanks to all the patients who participated in this study.

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Table

Table 1. Selection pressure analysis of the structural protein of DENV-2 (n = 202) using FEL, IFEL, MEME, and FUBAR.

Serial number	AA position	FEL		IFEL		MEME		FUBAR	
		ω	p-Value	ω	p-Value	ω	p-Value	ω	Posterior Prob(α□β)
1	11	/	/	/	/	□100	0.083	/	/
2	19	/	/	/	/	□100	0.027	/	/
3	35	/	/	/	/	□100	0.066	0.132	0.963
4	207	/	/	/	/	□100	0.061	0.174	0.916
5	209	0.049	0.000	0.114	0.012	□100	0.078	0.068	1.000
6	228	/	/	/	/	□100	0.056	/	/
7	332	/	/	/	/	□100	0.032	/	/
8	400	/	/	/	/	□100	0.004	/	/
9	451	0.070	0.014	0.000	0.011	□100	0.069	0.082	0.998
10	463	/	/	/	/	□100	0.037	/	/
11	464	0.164	0.008	0.000	0.001	□100	0.000	0.182	0.988
12	474	/	/	/	/	□100	0.089	/	/
13	475	0.153	0.078	0.000	0.047	□100	0.053	0.130	0.983
14	488	/	/	/	/	□100	0.053	0.164	0.928
15	506	/	/	/	/	□100	0.052	/	/
16	521	/	/	/	/	□100	0.053	0.133	0.976

Note: Criteria to consider sites with significant evidence of positive selection: p-value <0.1 in FEL, IFEL, and MEME, Posterior Prob (α<β) >0.9 in FUBAR, and omega should be greater than 1. Sites that were found to be positive by at least one method are included in the list. “/”: Represents that the site was not selected by the corresponding method as a positive or negative selection. “AA”: Represents amino acids.

Figures

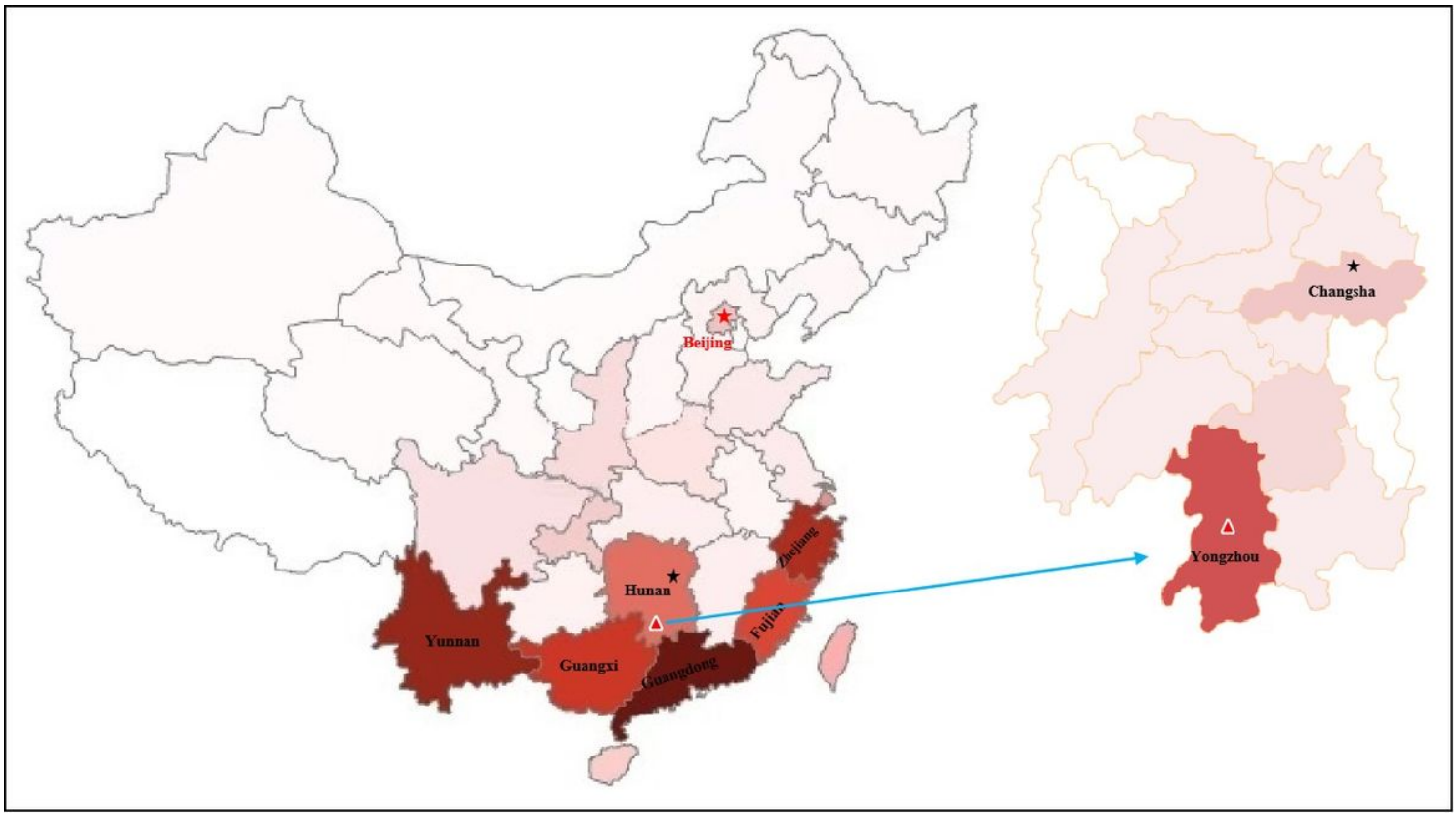

Figure 1

Geographic relationships between Hunan and other dengue outbreak areas in China. The intensity of the color in the figure depends on the amount of dengue cases, the darker the color is, the more dengue cases there are in the area, otherwise, the reverse. And the map of China shows the distribution of dengue cases in China in the past 15 years (from 2004 to 2018), the map of Hunan Province shows the distribution of dengue cases in 2018. Visible from the figure, Hunan Province is surrounded by areas with high incidence of dengue fever, such as Yunnan, Guangxi, Guangdong, Fujian, Zhejiang, Hainan and Taiwan. Moreover, the central area of this dengue fever outbreak is Qiyang County in Hunan, which is the most closely location in Hunan adjacent to Guangxi and Guangdong in geographically. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

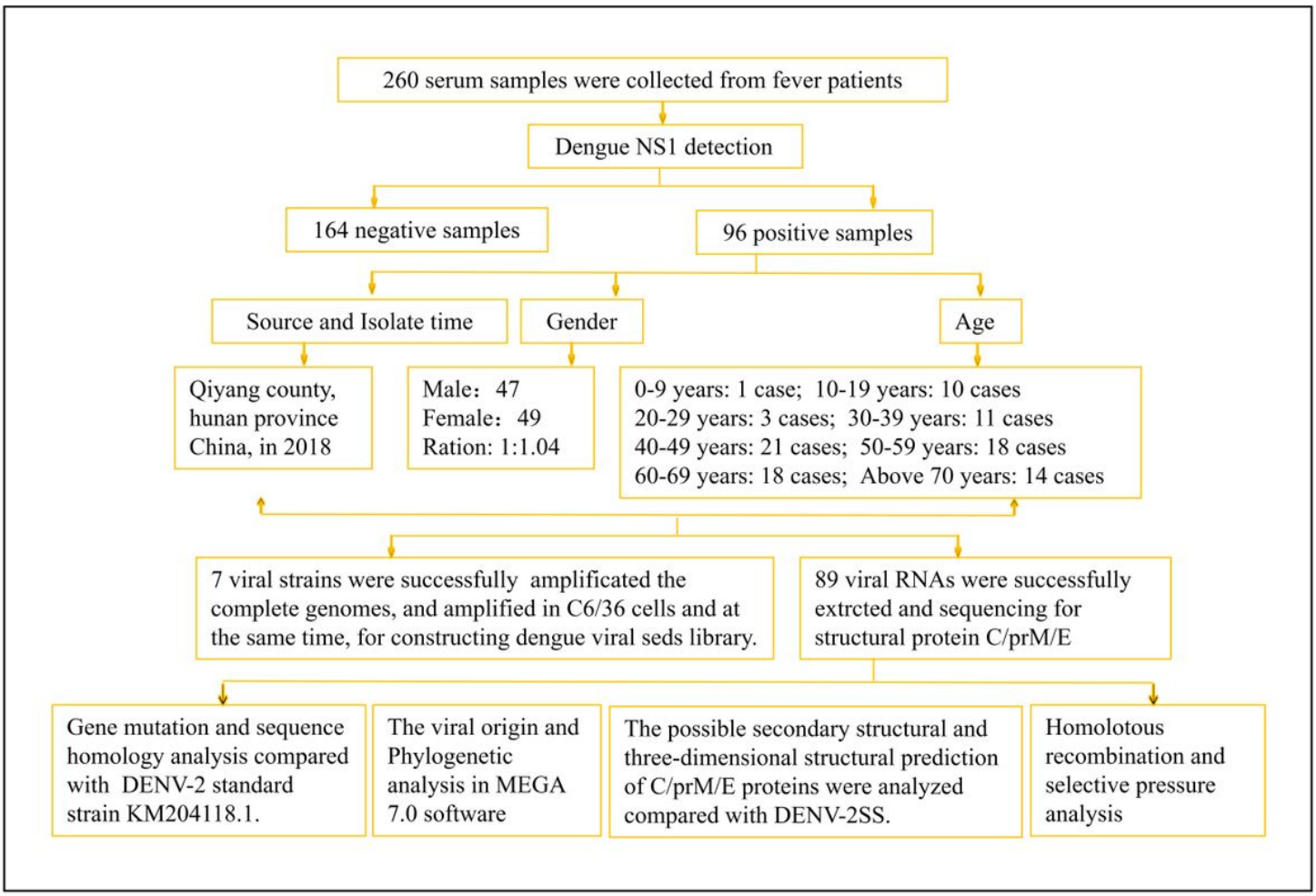

Figure 2

The study design and the following disposition of study subject. Two hundred and sixty patients who went to the hospital for fever were recruited in our study; among them, 96 cases were identified as dengue NS1-positive. Of these, serum samples were collected for virus amplification and viral RNAs extraction. Phylogenetic analysis was then conducted to characterize the origin and prevalence of DENV in Qiyang, Hunan during 2018.

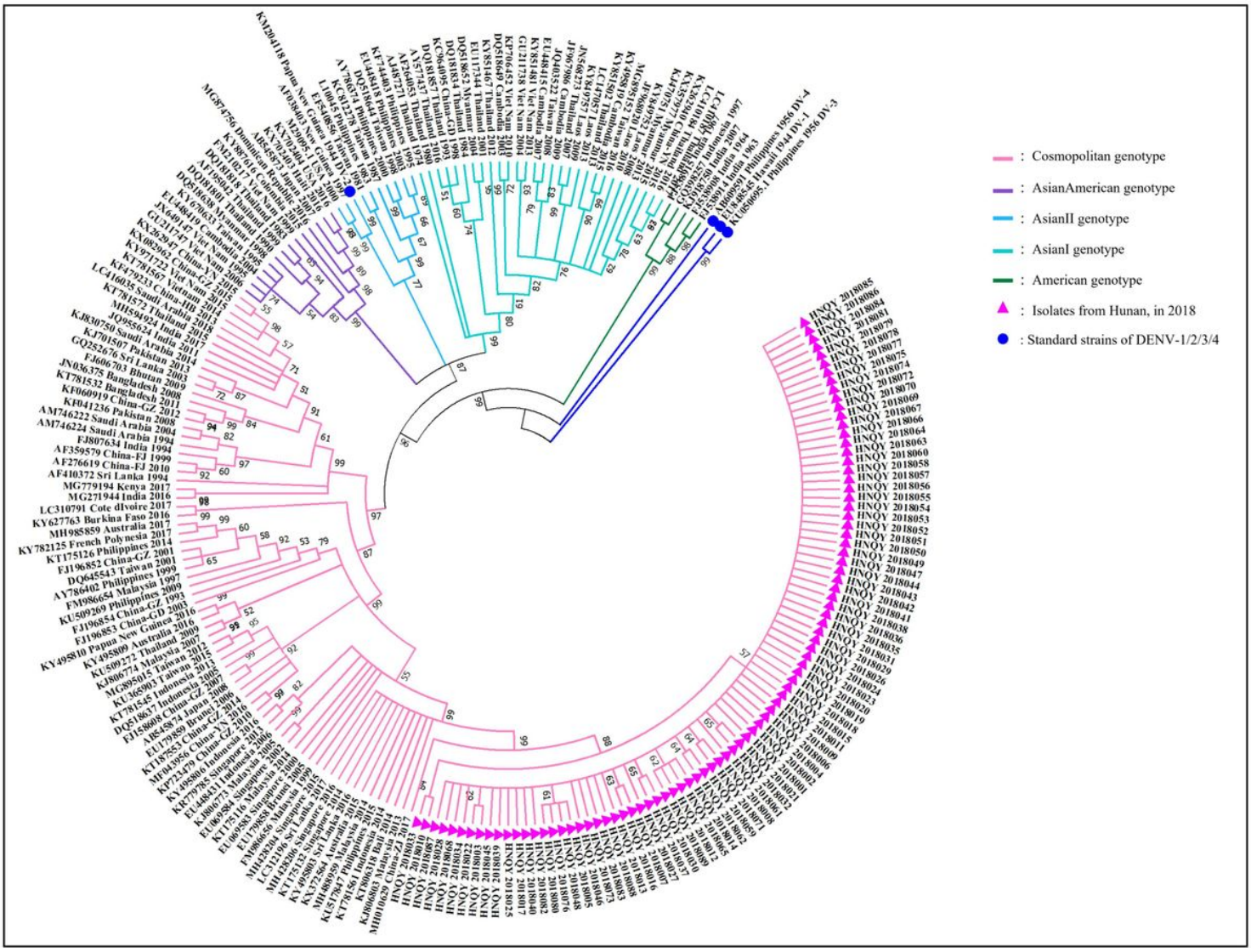

Figure 3

Phylogenetic tree of E protein of DENV-2 epidemic strains in Hunan Province, China, in 2018. The phylogenetic trees were constructed by the maximum-likelihood method with a Kimura 2 parameter model using MEGA 7.0 software (https://www. megasoftware. net). The rose red triangles in the picture represent 89 epidemic strains of Hunan; the blue dots represent the standard strains (DENV-1/2/3/4).

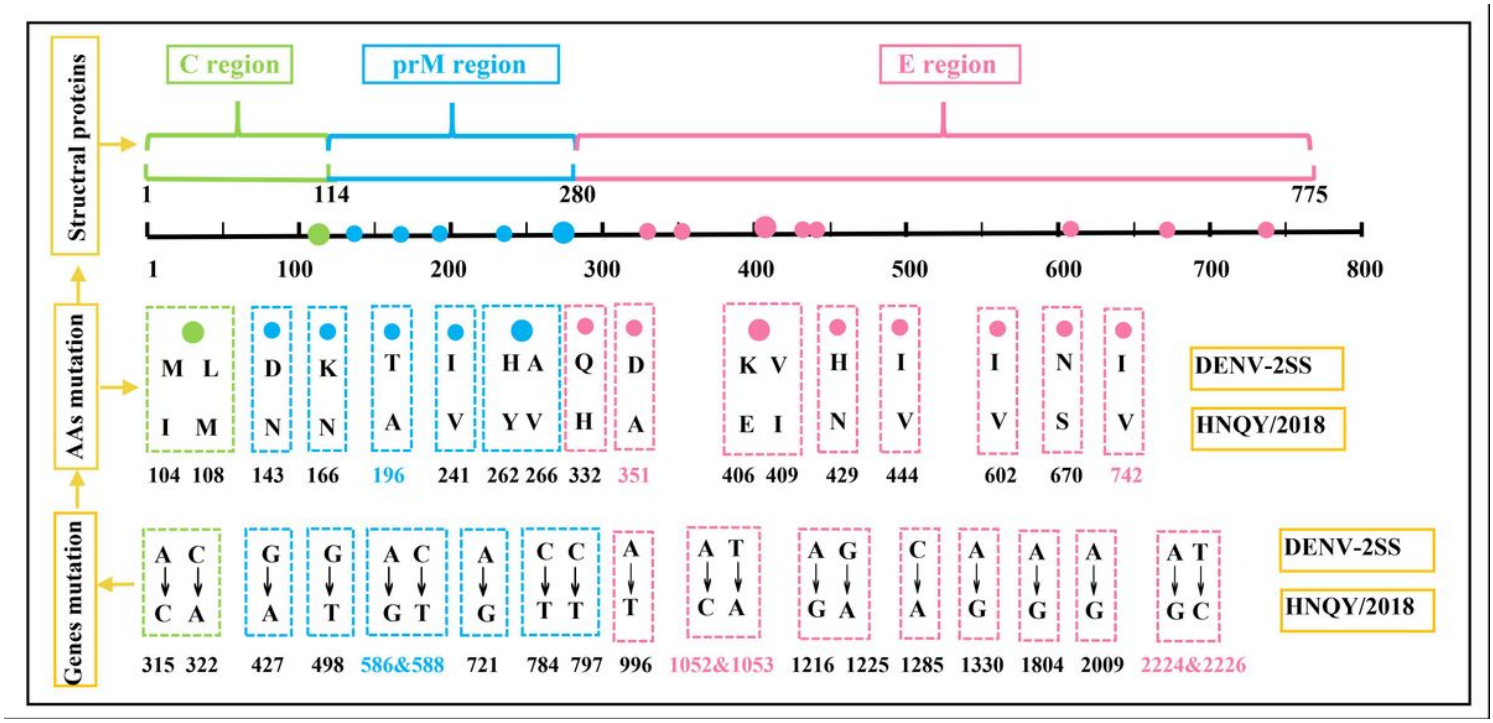

Figure 4

The gene and amino acid mutation site map of structural proteins of epidemic strains from Hunan (HNQY2018001-2018089) compared to the DV2 standard strain KM204118.1.

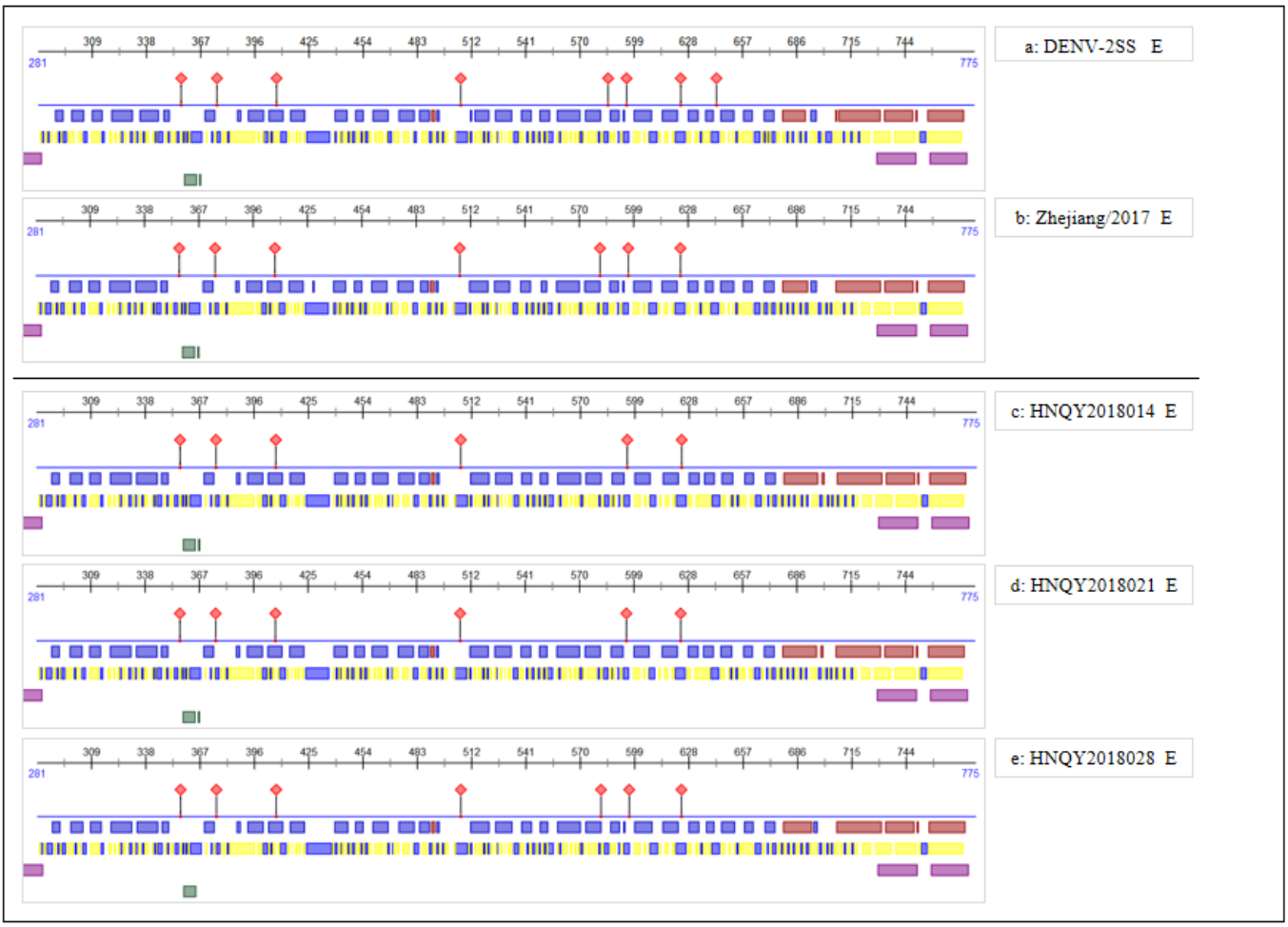

Figure 5

Prediction results of protein secondary structure of Hunan epidemic strains, DENV-2SS (KM204118) and Zhejiang/2017 (MG356770). Note: The red rhombus denotes the protein-binding region, the yellow dot denotes the DNA-binding region, and the purple dot denotes the RNA-binding region. Blue and red in the first line represent the strand and helix regions, respectively. Blue and yellow in the second line represent the exposed and buried regions, respectively. Purple in the third line indicates the helical transmembrane regions, and green in the fourth line represents the disordered regions.

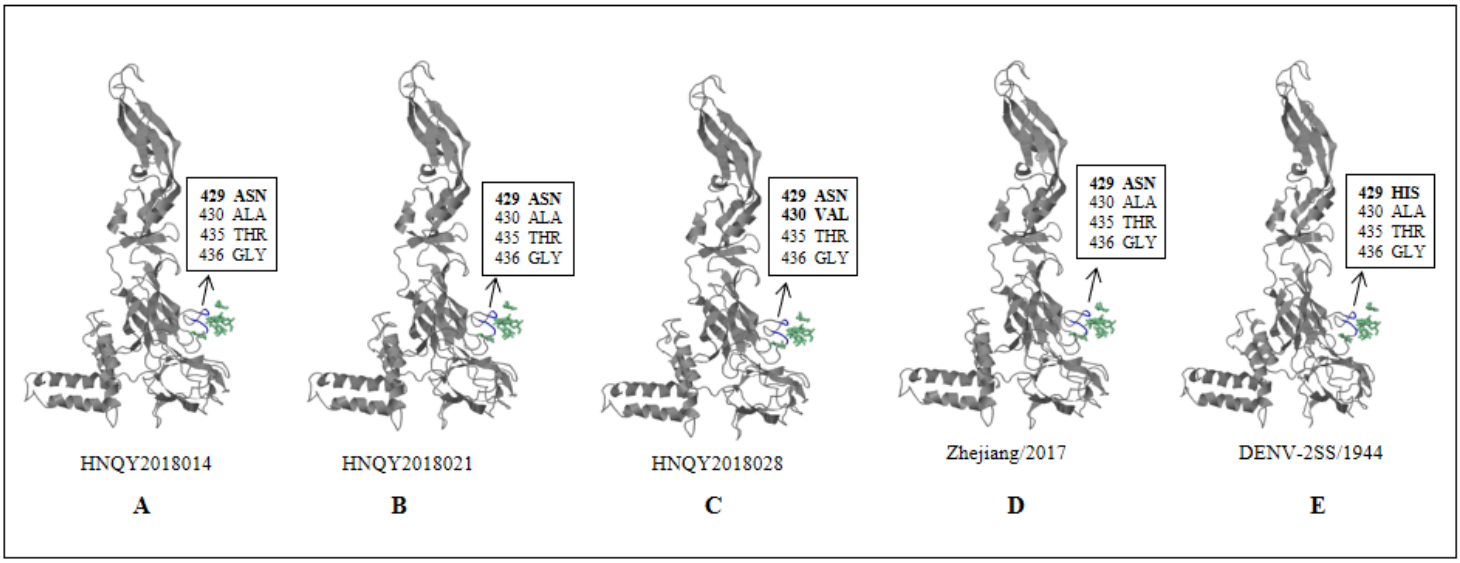

Figure 6

Predicted possible three-dimensional structure of structural protein E genes of three representative 2018 Hunan epidemic strains (HNQY2018014, 021, and 028), the closest strain (Zhejiang/2017, MH010629), and DENV-2 standard strain (DENV2-SS, KM204118). Blue indicates predicted protein binding sites. There are 4 possible binding sites in the E protein region of the five strains.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable2.doc
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- SupplementaryTable1.doc
- SupplementaryFigure2.doc

- SupplementaryTable3.doc