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BRIEF REPORT

Evidence of diversification of dengue virus type 3 genotype III in the South American region

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Abstract In order to gain insight into the genetic variability of dengue virus type 3 (DENV-3) genotype III isolated in the Latin American region, phylogenetic analysis were carried out using envelope (E) gene sequences from 57 DENV-3 genotype III strains isolated in 11 Latin American countries. At least six different genotype III clades were observed. Amino acids substitutions were found in domain III E protein neutralization epitopes and in surface-exposed domain II and III E protein amino acid sequences.

Nucleotide sequence data reported are available in the EMBL database under accessions numbers FM246466 through FM246471 and FM246473.

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Dengue virus (DENV) is a member of the genus *Flavivirus* of the family *Flaviviridae*. DENVs are mosquito-borne flaviviruses with a single-stranded, nonsegmented, positive-sense RNA genome of approximately 11 kb in length [34]. Dengue viruses are comprised of four distinct serotypes (DENV1 through DENV4), which are transmitted to humans through the bites of two mosquito species: *Aedes aegypti* and *Aedes albopictus* [7].

DENV causes a wide range of diseases in humans, from the acute febrile illness dengue fever (DF) to life-threatening dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). DENV has become endemic to many Latin American countries in the last 25 years [40]. Just in Brazil, a total of 4,243,049 dengue cases have been reported between 1981 and 2006, including 5,817 cases of DHF/DSS [29].

Based on sequences of the complete envelope (E) gene, and using a cut-off of 6% divergence, each DENV serotype can be divided in different genotypes [35]. In the case of DENV-3, this serotype has been divided into four genotypes (I–IV) [16, 21, 25], sometimes including a genotype V [8].

The emergence of DHF in Sri Lanka in 1989 coincided with the appearance there of a new DENV-3 genotype III variant, which spread from the Indian subcontinent into Africa and Latin America [25]. In 1998/1999, DENV-3 genotype III was introduced into Caribbean countries such as Puerto Rico, Barbados, Jamaica, and Martinique, and finally in 2000, into South America [2–4, 25, 30–33, 42].

Despite that the Sri Lankan DENV-3 genotype III and associated American isolates have been linked to severe disease epidemics [38], little is known about the degree of genetic variability among DENV-3 genotype III isolates circulating in the South American region.

In order to gain insights into these matters, we first obtained serum samples from 23 Ecuadorian patients presenting dengue-like syndromes from Instituto Nacional de Higiene y Medicina Tropical "Leopoldo Izquieta Perez" at Guayaquil, Ecuador. All samples from these patients were found to be positive for dengue infection by the presence of immunoglobulin M (IgM), elevation of specific IgG, or both, using an "in-house" dengue virus-specific enzyme-linked immunosorbent assay (ELISA IgM Capture). In order to assign each Ecuadorian DENV strain to an appropriate serotype, serum samples from patients who tested positive in the serology assays underwent reverse transcription-PCR (RT-PCR), using a multiplex PCR method according to Harris et al. [14]. By these means, it was possible for us to assign 9 of the 23 Ecuadorian strains to the DENV-3 serotype (not shown). Then, serum samples from patients assigned to DENV-3 underwent RT-PCR according to Aquino et al. [2] in order to obtain amplicons containing the full-length sequences from the DENV envelope (E) gene. To avoid false positive results, the recommendations of Kwok and Higuchi [20] were strictly adhered to. Amplicons were purified using a QIAquick PCR Purification Kit from QIAGEN, according to instructions from the manufacturers. The sequence reaction was carried out using the Big Dye DNA sequencing kit (Perkin–Elmer) on a 373 DNA sequencer apparatus (Perkin–Elmer). Both strands of the PCR product were sequenced in order to avoid discrepancies. Envelope (E) gene nucleotide sequences from the DENV-3 Ecuadorian strains, corresponding to position 1014 through 2413 of the DENV genome (relative to the sequence of DENV-3 strain NC001475) were obtained. These sequences were deposited in the EMBL database under accession numbers FM246466 through FM246473, (see also Supplementary Material Table 1).

To study the degree of genetic variability of DENV-3 genotype III strains isolated in the Latin American region, the DENV-3 E gene sequences obtained from the Ecuadorian strains were aligned with 48 comparable sequences of DENV-3 from strains isolated in 11 different Latin American countries, the genotypes of which have been described previously, as well as with 20 sequences from all DENV-3 genotypes isolated elsewhere, using the CLUSTAL W program [41]. For sequence names, country of isolation, dates and accession numbers, see Supplementary Material Table 1. All these sequences were obtained by the use of the Flavitrack database [26] (available at: http://carnot.utmb.edu/flavitrack/resultIndex.php).

Once aligned, software from the Datamonkey Webserver [19] (available at: http://www.datamonkey.org) was used to identify the optimal evolutionary model for the sequence dataset. Akaike Information Criteria and the Ln-likelihood indicated that the GTR model best fit the sequence data. Using this model, maximum-likelihood trees were constructed using software from the PhyML program [13] (available at: http://www.phylogeny.fr/phylo_cgi/phyml).

As a measure of the robustness of each node, we employed an approximate Likelihood Ratio Test (aLRT), which assesses whether the branch being studied provides a significant likelihood gain in comparison with the null hypothesis, which involves collapsing that branch but leaving the rest of the tree topology identical [1]. aLRT calculations were done using three different approaches: (a) by using the minimum of χ^2-based calculations; (b) by a Shimodaira-Hasegawa-like procedure (SH-like), which is non-parametric, and (c) by a combination of both (SH-like and the minimum χ^2-based calculations), which is the most conservative option for these calculations [1]. We have also used the bootstrap method (100 replications), as implemented in the PhyML program [13]. No significant differences were observed in the results found using these four different approaches. The results of these studies are shown in Fig. 1.

All strains in the tree cluster according to their genotype. Genotype III strains cluster together, strains belonging to the other DENV-3 genotypes cluster separately. These clusters are supported by very high aLRT values, (see Fig. 1).

Inside the genotype III cluster of strains isolated in Latin America, different genetic clades can be observed. Four clades, composed of strains isolated in Argentina, Venezuela and Puerto Rico, can be observed. Most of these clusters are supported by very high aLRT values (Fig. 1, bottom). Besides these clades, strains circulating in Latin American countries appear to group in two distinct genetic groups: one exclusively composed of all Ecuadorian and Peruvian strains and strains isolated in Cuba and Puerto Rico (see Fig. 1, top), and another exclusively composed of all strains isolated in Bolivia, Brazil and Paraguay (see Fig. 1, middle). In order to gain insight into these matters, the same phylogenetic studies were repeated using all strains isolated in those countries, using strain L11427 (genotype I) as an outgroup. The results of these studies are shown in Supplementary Material Fig. 3. Two distinct genetic groups are clearly observed, in agreement of very recent reports [18]. The results of these studies show that at least six different genotype III genetic clades have been circulating in Latin America.

In order to gain insight into the diversification of DENV-3 genotype III in the Latin American region, envelope (E) gene sequences from strains representative of different genotype III clades, as well as DENV-3 sequences from strains belonging to different genotypes and isolated

Fig. 1 Maximum-likelihood phylogenetic tree analysis of DENV-3 strains isolated in Latin America. Strains in the trees are shown by their accession number for previously described strains, followed by their geographic location and year of isolation in *parentheses*. Ecuadorian strains are shown by *name*, in *italics*, and indicated by an *arrow*. The strain's genotype is indicated on the right side of figure or in *parentheses*. For strain names and year of isolation, see Supplementary Material Table 1. Numbers at the branches show aLRT values, calculated using a Shimodaira-Hasegawa-like procedure (SH-like). For results found using the minimum of χ^2-based calculations, a combination of both methods (SH-like and the minimum χ^2-based calculations), or by using the bootstrap method, see Supplementary Material Fig. 2. The *bar* at the bottom of the figure denotes distance. Clades found in genotype III strains circulating in the Latin American region are highlighted in *gray*

elsewhere, were aligned with corresponding sequences from DENV-3 strain CH53489 (accession No. M86733), for which the structure of the E protein (residues 1-393) has been determined by X-ray crystallography [27] (Protein Data Bank accession code: 1uzg), using the CLUSTAL W program [41]. This strain belongs to DENV-3 genotype II (see Supplementary Material Fig. 1). Once aligned, using the MEGA 3 program [20], sequences were translated into amino acids. The results of these studies are shown in Fig. 2.

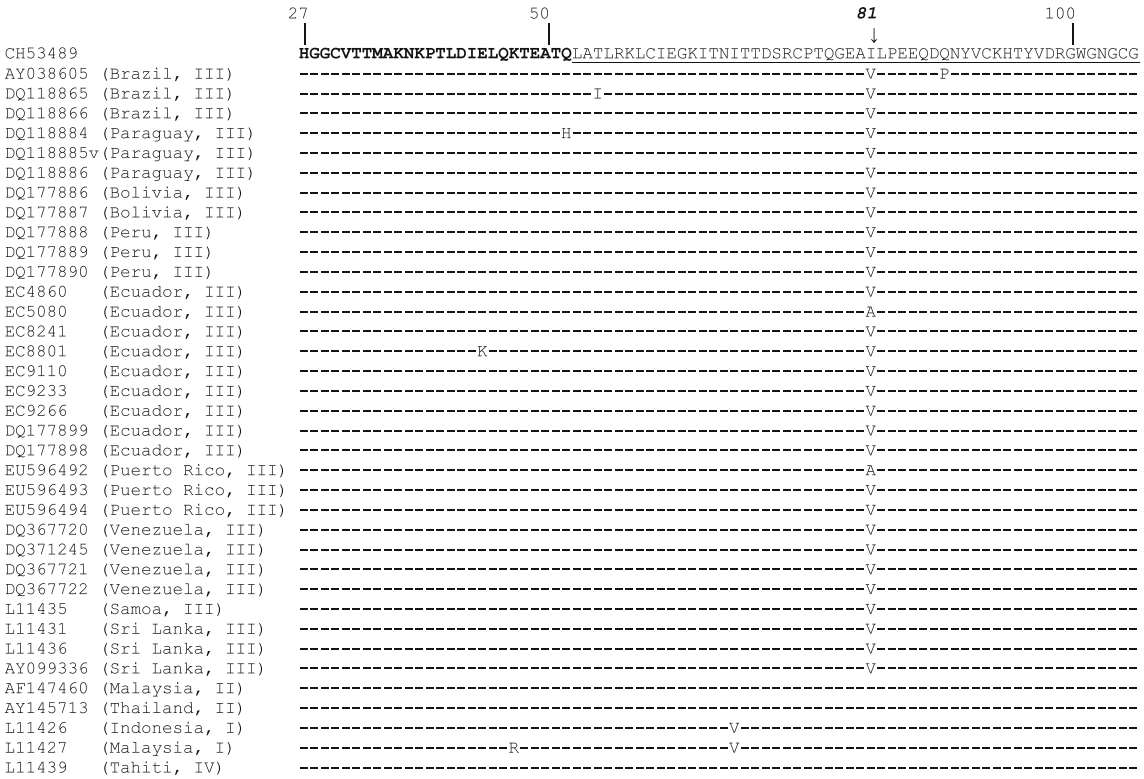


Fig. 2 Alignment of DENV-3 E protein amino acid sequences. Previously described strains are listed by accession number for strains on the left side of the figure, followed by their geographic location of isolation and genotype in *parentheses*. Ecuadorian strains are listed by name. Identity to the CH53489 strain amino acid sequence is indicated by a *dash*. Amino acids positions (relative to strain CH53489) are shown by numbers at the top of the alignment. Sequences corresponding to E protein domains I, II and III are shown in *bold*, *underlining* and *italics*, respectively. Surface-exposed sequences previously identified on the dimeric DENV-3 E protein are shown in gray [9]. Potential ELK/KLE-type and KELK/KLEK-type motifs are shown in *italics*, *bold* and *underlined* [9]

DENV-3 E protein resembles its homolog of DENV-2 in its dimeric structure and in the details of its protein folding [28]. Each monomer consists of three domains: domain I, an eight-stranded β-barrel, which organizes the structure; domain II, which contains 12 β-strands and two α-helices; and domain III, which is an IgC-like domain, with 10 β-strands. In solution and in the crystals, two monomers of E assemble head to tail to form a dimer [27].

As it can be seen in Fig. 2, different amino acid changes shared by all genotype III strains can be observed in all E protein domains. Interestingly, amino acid changes in position 329 are only observed in strains of genotype III isolated in Peru, Ecuador and Venezuela. This is also shared with a genotype II strain (see Fig. 2). This alanine (A)-to-valine (V) substitution implies a change of a hydrophobic amino acid by another hydrophobic but aliphatic amino acid. Regarding the putative effect of this change on the structure, V has an additional property that might be considered: while most of the amino acids contain only one non-hydrogen substituent attached to their C-beta carbon, V contains two. This means that there is a lot more bulkiness near the protein backbone. Whether this may relate to escape of immune recognition or neutralization remains to be established.

Amino acids substitutions shared by all genotype III strains can also be observed at positions 301 and 383. These changes are non-conservative, since substitution at position 301 involves a change of leucine (L) (a hydrophobic aliphatic non-polar amino acid) to threonine (T) (a polar amino acid), while substitution at position 383 involves a change of lysine (K) (a positive charged polar amino acid) to asparagine (N) (an uncharged polar amino acid) (see Fig. 2). Moreover, amino acids substitutions at these positions have been previously reported to be involved in neutralization epitopes [15, 17].

An asparagine (N) at position 388 was found in all strains included in these studies (see Fig. 2). Previous studies have shown that an asparagine at this position appears to cause increased incidences of hemorrhagic fever [22].

In order to map the amino acids substitutions on the DENV-3 E protein structure, and to study the differences

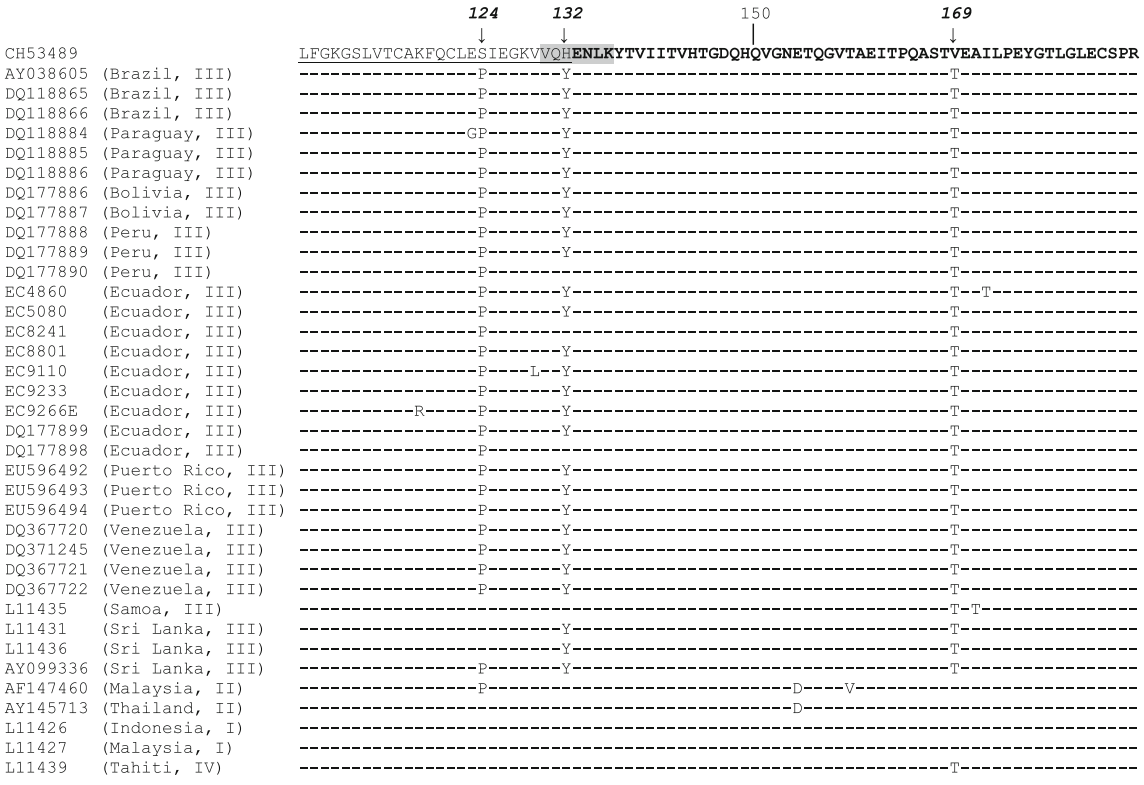
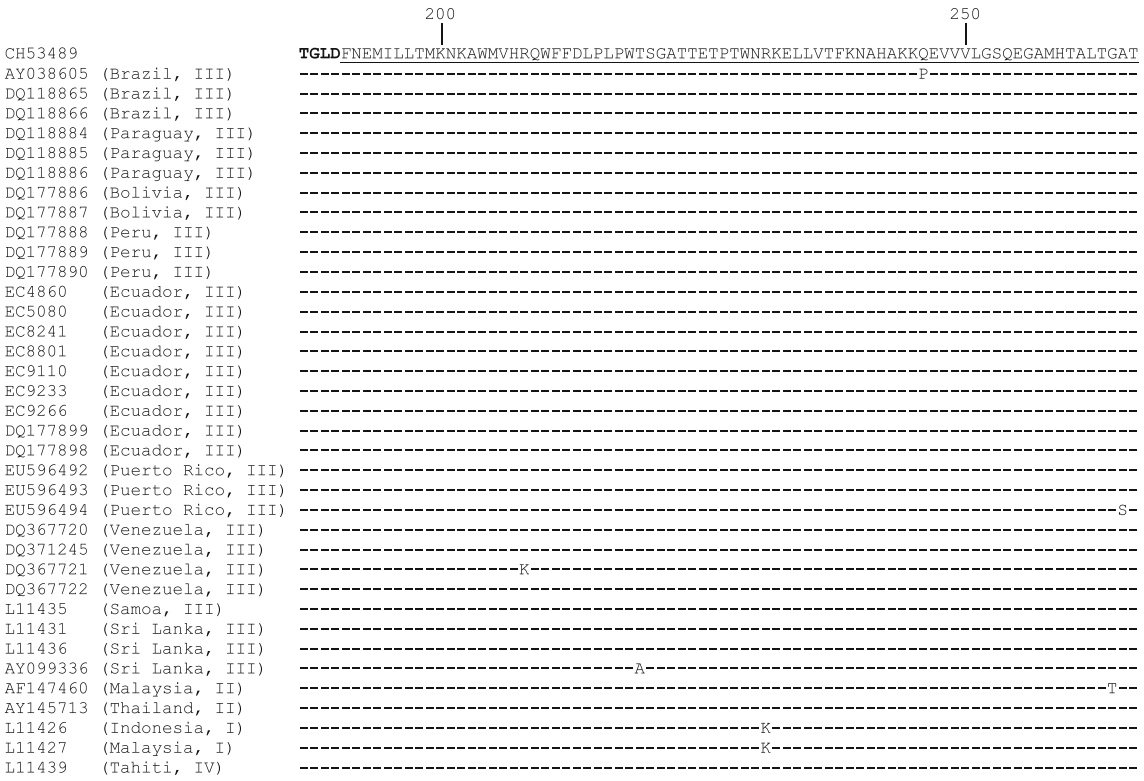


Fig. 2 continued



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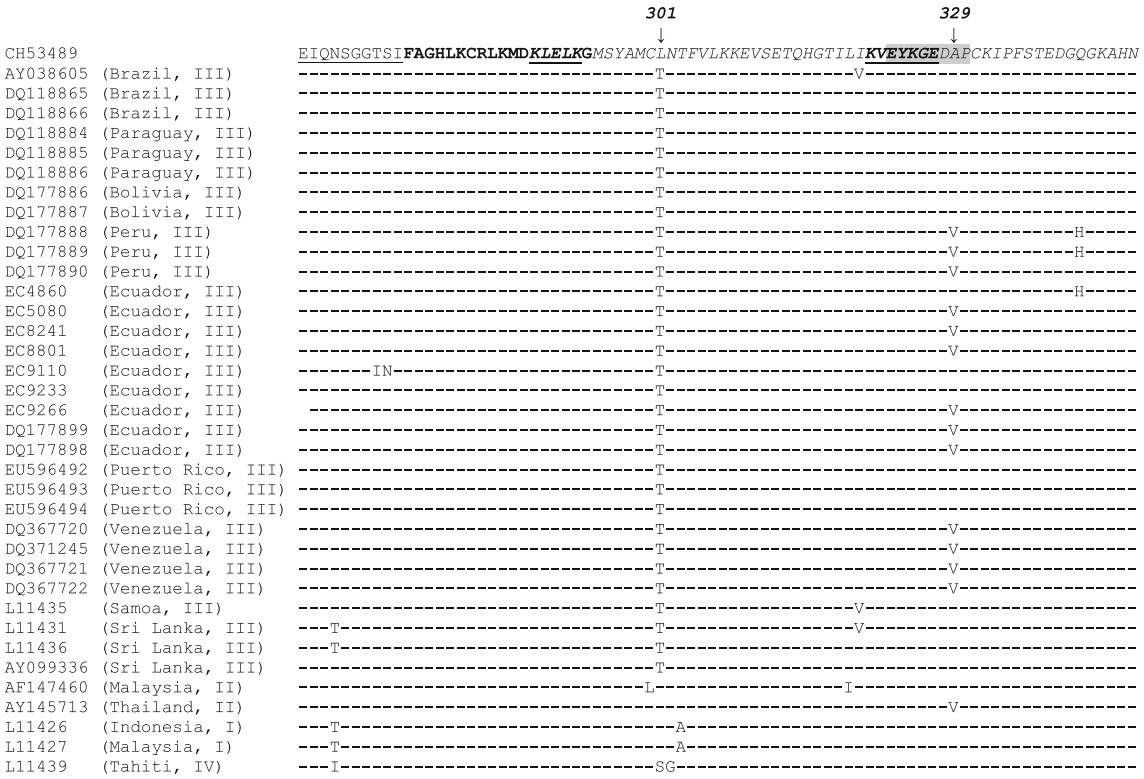

Fig. 2 continued

found among DENV-3 genotype III E proteins and the E protein structure of CH53489 strain, we employed An Interactive Server-side Molecule Image Generator (AISMIG) [6]. The results of these studies are shown in Fig. 3.

Amino acid substitution at position 329 of domain III, found in E proteins from strains isolated in Ecuador, Peru and Venezuela, is situated in previously identified surface-exposed amino acids in the DENV-3 E glycoprotein [27, 28] (see Fig. 3).

The amino acid substitution at position 132 of domain II observed in DENV-3 genotype III E protein sequences is also situated in surface-exposed amino acids of the DENV-3 E protein (see Fig. 3) [9].

Recent and older studies have shown that escape mutants that map to serotype-specific residues are not evenly distributed throughout the E protein structure; instead, they lie exclusively in domain III [5, 11, 12, 23, 24, 27, 39].

Very recent studies have shown that domain III of the E protein is recognized by monoclonal antibodies raised against nonstructural-1 (NS1) DENV-2 protein [9]. This may have important consequences for the pathogenesis caused by DENV, since human immunoglobulin G (IgG) polyclonal antibodies (PAbs) generated against DENV NS1 protein is detected only during the convalescent phase in primary DENV infections but it is strongly identified during the acute phase of secondary DENV infections [37], indicating that they may play a role in DHF/DSS. During DENV infection, human PAb responses are generated to multiple acidic (E or D), aliphatic/aromatic (G, A, I, L or V/F, W, or Y), or basic (K or R) amino acids or ELK- or KELK-type motifs present in either orientation on the DENV NS1 protein, and these responses are higher in DSS patients than in patients with mid disease [10]. The location of these epitopes on the DENV-3 E protein may help to explain their pathogenic capacities through increased antibody-enhancement replication of DENV [9].

The localization of all these mutations in domain III is significant, because domain III have been proposed to bind to cell-surface receptor [27]. The most likely mechanism of neutralization by antibodies recognizing epitopes on domain III is inhibition of cellular attachment. Nevertheless, studies have suggested that inhibition of viral attachment by these antibodies is relatively low and that other factors may contribute to neutralization [36].

The results of these studies revealed the presence of at least six different genetic clades of DENV-3 genotype III circulating in the Latin American region (Fig. 1). Although

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                              350                                    383  388                402
                                |                                     ↓    |                  |
CH53489                      GRLITANPVVTKKEEPVNIEAEPPFGESNIVIGIGDKALKINWYKKGSSIGKMFEA
AY038605 (Brazil, III)       ------------------------------------N-------------------
DQ118865 (Brazil, III)       ------------------------------------N-------------------
DQ118866 (Brazil, III)       ---------------------------------T--N-------------------
DQ118884 (Paraguay, III)     ---------------------------------T--N-------------------
DQ118885 (Paraguay, III)     ------------------------------------N-------------------
DQ118886 (Paraguay, III)     ---------------------------------T--N-------------------
DQ177886 (Bolivia, III)      ---------------------------------T--N-------------------
DQ177887 (Bolivia, III)      ---------------------------------T--N-------------------
DQ177888 (Peru, III)         ------------------------------------N-------------------
DQ177889 (Peru, III)         ------------------------------------N-------------------
DQ177890 (Peru, III)         ------------------------------------N-------------------
EC4860   (Ecuador, III)      ------------------------------------N-------------------
EC5080   (Ecuador, III)      ------------------------------------N-------------------
EC8241   (Ecuador, III)      -----GS-----------------------------N-------R-----------
EC8801   (Ecuador, III)      ------------------------------------N-------------------
EC9110   (Ecuador, III)      ------------------------------------N-------------------
EC9233   (Ecuador, III)      ------------------------------------N-------------------
EC9266   (Ecuador, III)      ------------------------------E-----N-------------------
DQ177899 (Ecuador, III)      ------------------------------------N-------------------
DQ177898 (Ecuador, III)      ------------------------------------N-------------------
EU596492 (Puerto Rico, III)  ------------------------------------N-------------------
EU596493 (Puerto Rico, III)  ------------------------------------N-------------------
EU596494 (Puerto Rico, III)  ------------------------------------N-------------------
DQ367720 (Venezuela, III)    ------------------------------------N-------------------
DQ371245 (Venezuela, III)    ------------------------------------N-------------------
DQ367721 (Venezuela, III)    ------------------------------------N-------------------
DQ367722 (Venezuela, III)    ------------------------------------N-------------------
L11435   (Samoa, III)        ---------------------------------T--N-------------------
L11431   (Sri Lanka, III)    ------------------------------------N-------------------
L11436   (Sri Lanka, III)    ---------------------------------T--N-------------------
AY099336 (Sri Lanka, III)    ------------------------------------N-------------------
AF147460 (Malaysia, II)      --------------------------------I--K--------------------
AY145713 (Thailand, II)      --------------------------------------------------------
L11426   (Indonesia, I)      --------------------------------------------------------
L11427   (Malaysia, I)       --------------------------------------------------------
L11439   (Tahiti, IV)        ---------------------------------T-----R----------------
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Fig. 2 continued

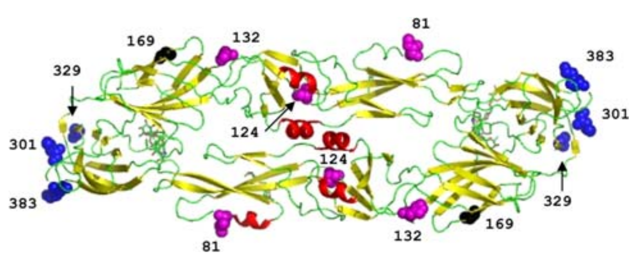

Fig. 3 Structure of the protein E dimer of DENV-3. Residues changes found in DENV-3 genotype III are shown in a space-filling representation in *black*, *magenta* and *blue* for changes in domains I, II and III, respectively, and their amino acids sequence positions are shown by *numbers*

a high degree of genetic variation has been observed, the E protein of these strains is relatively well conserved among all clades (Fig. 2). Nevertheless, changes in specific amino acids positions, particularly in protein E domain III or in surface-exposed amino acids may have accounted for these strains escaping neutralization and may help to explain, at least in part, their particular phenotype characteristics (see Figs. 2, 3).

More studies will be needed to characterize DENV-3 genotype III strains circulating in all regions of the world. This will permit to design the more appropriate anti-viral strategies against this important pathogen.

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References

1. Anisimova M, Gascuel O (2006) Approximate likelihood ratio test for branchs: a fast, accurate and powerful alternative. Syst Biol 55:539–552
2. Aquino VH, Anatriello E, Goncalvez PF et al (2004) Molecular epidemiology of Dengue Type 3 Virus in Brazil and Paraguay, 2002–2004. Am J Trop Med Hyg 75:710–715
3. Balmaseda A, Sandoval E, Perez L et al (1999) Application of molecular typing techniques in the 1998 dengue epidemic in Nicaragua. Am J Trop Med Hyg 61:893–897
4. Barrero PR, Mistchenko AS (2008) Genetic analysis of dengue virus type 3 isolated in Buenos Aires, Argentina. Virus Res 135:83–88
5. Beasley DW, Aaskov JG (2001) Epitopes on the dengue 1 virus envelope protein recognized by neutralizing IgM monoclonal antibodies. Virology 279:447–458
6. Bohne-Lang A, Groch WD, Ranziger R (2005) AISMIG: an interactive server-side molecule image generator. Nucleic Acids Res 33(Web Server issue):705–709
7. Clyde K, Kyle JL, Harris E (2006) Recent advances in deciphering viral and host determinants of Dengue virus replication and pathogenesis. J Virol 80:11418–11431
8. Diaz FJ, Black WC, Farfan-Ale JA et al (2006) Dengue virus circulation and evolution in Mexico: a phylogenetic perspective. Arch Med Res 37:760–773
9. Falconar AKI (2008) Monoclonal antibodies that bind to common epitopes on the Dengue Virus Type 2 nonstructural-1 and Envelope Glycoproteins display weak neutralizing activity and differentiated responses to virulent strains: implications for pathogenesis and vaccines. Clin Vaccine Immunol 15:549–561
10. Falconar AKI (2007) Antibody responses to immunodominant ELK-type motifs on the dengue virus nonstructural-1 glycoprotein during live dengue virus infections in mice and humans: implications for diagnosis, pathogenesis, and vaccine design. Clin Vaccine Immonol 15:493–504
11. 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 82:8828–8837
12. Gromowski GD, Barrett AD (2007) Characterization of an antigenic site that contains a dominant, type-specific neutralization determinant on the envelope protein domain III (ED3) of dengue 2 virus. Virology 366:349–360
13. Guindon S, Lethiec F, Duroux P et al (2005) PHYML Online—a web server for fast maximum likelihood-based phylogenetic inference. Nucleic Acids Res 33(Web Server issue):W557–W559
14. Harris E, Roberts TG, Smith L (1998) Typing of dengue viruses in clinical specimens and mosquitoes by single-tube multiplex reverse transcriptase PCR. J Clin Microbiol 36:2634–2639
15. Hiramatsu K, Tadano M, Men R (1996) Mutational analysis of a neutralization epitope on the dengue type 2 virus (DEN2) envelope protein: monoclonal antibody resistant DEN2/DEN4 chimeras exhibit reduced mouse neurovirulence. Virology 224:437–445
16. Holmes EC, Twiddy SS (2003) The origin, emergence and evolutionary genetics of dengue virus. Infect Genet Evol 3:19–28
17. Hung JJ, Hsieh MT, Young MJ et al (2004) An external loop region of domain III of dengue virus type 2 envelope protein is involved in serotype-specific binding to mosquito but not mammalian cells. J Virol 78:378–388
18. Kochel T, Aguilar P, Felices V et al (2008) Molecular epidemiology of dengue virus type 3 in Northern South America: 2000–2005. Infect Genet Evol 8:682–688
19. Kosakovsky-Pond S, Frost SD (2005) Datamonkey: rapid detection of selective pressure on individual sites of codon alignments. Bioinformatics 21:2531–2533
20. Kumar S, Tamura K, Nei M (2004) MEGA 3: Integrated software for molecular evolution genetics analysis and sequence alignment. Brief Bioinformatics 5:150–163
21. Lanciotti RS, Lewis JG, Gubler DJ et al (1994) Molecular evolution and epidemiology of dengue-3 viruses. J Gen Virol 75:65–75
22. Leitmeyer KC, Vaughn DW, Watts DM et al (1999) Dengue virus structural differences that correlate with pathogenesis. J Virol 73:4738–4747
23. Lin B, Parrish CR, Murray JM et al (1994) Localization of a neutralizing epitope on the envelope protein of dengue virus type 2. Virology 202:885–890
24. Lisova O, Hardy F, Petit V et al (2007) Mapping to completeness and transplantation of a group-specific, discontinuous, neutralizing epitope in the envelope protein of dengue virus. J Gen Virol 88:2387–2397
25. Messer WB, Gubler DJ, Harris E et al (2003) Emergence and global spread of a dengue serotype 3, subtype III virus. Emerg Infect Dis 9:800–809
26. Misra M, Schein CH (2007) Flavitrack: an annotated database of flavivirus sequences. Bioinformatics 23:2645–2647
27. Modis Y, Ogata S, Clements D et al (2005) Variable surface epitopes in the crystal structure of dengue virus type 3 envelope glycoprotein. J Virol 79:1223–1231
28. Modis Y, Harrison SC (2003) A ligand-binding pocket in the dengue virus envelope glycoprotein. Proc Natl Acad Sci USA 100:6986–6991
29. Nogueira RM, de Araujo JM, Schatzmayr HG (2007) Dengue viruses in Brazil, 1986–2006. Rev Panam Salud Publica 22:358–363
30. Nogueira RMR, Miagostovich MP, Filippis AMB et al (2001) Dengue virus type 3 in Rio de Janeiro, Brazil. Mem Inst Oswaldo Cruz 96:925–926
31. Peyrefitte CN, Pastorino BAM, Bessaud M et al (2005) Dengue type 3 virus, Saint Martin, 2003–2004. Emerg Infect Dis 11:757–761
32. Peyrefitte CN, Coussinier-Paris P, Mercier-Perennec V et al (2003) Genetic characterization of newly reintroduced dengue virus type 3 in Martinique (French West Indies). J Clin Microbiol 41:5195–5198
33. Regato M, Recarey R, Moratorio G et al (2008) Phylogenetic analysis of the NS5 gene of dengue viruses isolated in Ecuador. Virus Res 132:197–200
34. Rice CM (1996) Flaviviridae: the viruses and their replication. In: Fields BN, Knipe DM, Howley PM, Chanock RM, Melnick JL, Monath TP (eds) Virology. Lippincott-Raven, Philadelphia, pp 931–1034
35. Rico-Hesse R (1990) Molecular evolution and distribution of dengue viruses type 1 and 2 in nature. Virology 174:479–493
36. Roehrig JT, Bolin RA, Kelly RG (1998) Monoclonal antibody mapping of the envelope glycoprotein of the dengue 2 virus, Jamaica. Virology 246:317–328
37. Shu PY, Chen LK, Chang SF et al (2003) Comparison of capture immuno-globulin M (IgM) and IgG enzyme-linked immunosorbent assay (ELISA) and non-structural protein NS1 serotype-specific IgG ELISA for differentiation of primary and secondary dengue virus infections. Clin Diagn Lab Immunol 10:622–630
38. Silva RL, de Silva AM, Harris E et al (2008) Genetic analysis of Dengue 3 virus subtype III 5′ and 3′ non-coding regions. Virus Res 135:320–325
39. Sukupolvi-Petty S, Austin SK, Purtha WE et al (2007) Type- and subcomplex-specific neutralizing antibodies against domain III of dengue virus type 2 envelope protein recognize adjacent epitopes. J Virol 81:12816–12826

40. Thomas SJ, Strickman D, Vaughn DW (2003) Dengue epidemiology: virus epidemiology, ecology and emergence. Adv Virus Res 61:235–289
41. Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acid Res 22:4673–4680
42. Uzcategui NY, Comach G, Camacho D et al (2003) Molecular epidemiology of dengue virus type 3 in Venezuela. J Gen Virol 84:1569–1575