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Short Communication

Mutant spectrum of dengue type 1 virus in the plasma of patients from the 2006 epidemic in South China

Zeng Tu [a,*], Yong-lin He [a], He Lu [a], Lei Xu [a], Zhi-bang Yang [a], Chun Yang [a], Wei-jun Chen [b]

[a] College of Basic Medical Sciences, Chongqing Medical University, Chongqing, China
[b] Beijing Genomics Institute, Chinese Academy of Sciences, Beijing, China

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SUMMARY

The aim of the present study was to explore the mutant spectrum of dengue type 1 virus (DENV-1) within individuals during the 2006 dengue epidemic in South China. A 513-bp fragment including most of domain III of the envelope (E) gene was amplified directly from clinical samples, then cloned and sequenced. A total of 89 clones from six patients (range 11–17 clones per patient) were sequenced. Genetic diversity was calculated using MEGA 4 package. The total number of nucleotide mutations was 113 (3.7%) within the sequenced 513-bp E gene, with a range of 15 (3%) to 24 (4.7%) within individual viral populations, harboring more non-synonymous than synonymous mutations. The extent of sequence diversity varied among patients, with the mean diversity ranging from 0.19% to 0.32%, and the mean pairwise p-distance ranging from 0.34% to 0.65%. No genome-defective virus was detected in any clone in this study. Purifying selection may be the main driving force for the intrahost evolution: the mean dN/dS ratio was 0.532. Our findings contribute to the understanding of the genetic variation of DENV-1 in South China.

1. Introduction

All four serotypes of dengue virus (denoted as DENV-1, -2, -3, and -4) have been isolated in South China.[1] DENV-1 is the dominant serotype, with the latest and most severe outbreak in 2006.[1,2] We have previously reported that DENV in China has shown an extensive genetic diversity and complex molecular evolution over the course of 30 years.[2] The aim of the present study was to address the mutant spectrum of DENV-1 within individuals during the 2006 dengue epidemic in mainland China.

2. Methods

Serum samples were collected from six Chinese patients with classic dengue fever who were living in Guangzhou during the epidemic in 2006, within 7 days after the onset of symptoms. All sera tested were positive for DENV-1 IgM by indirect immunofluorescence assay. Viral RNA extraction, reverse transcription, and PCR were carried out as previously reported.[2] A pair of primers (primer E1578F, 5′-ACTTACCACTGCCTTGGACCT-3′; primer E2132R, 5′-TCCCTATGGTGCTTCCTTTCT-3′) was designed to amplify a 513-bp fragment of the envelope (E) gene. Subsequent PCR products were cloned and transformed into *Escherichia coli* DH5α competent cells, and 11 to 17 clones from six patients (total 89 clones) were picked out for 3730 automated sequencer sequencing using the M13 primer. Sequences were then entered into MEGA 4 package for analysis of the mean diversity and the extent of sequence divergence among clones at the nucleotide and amino acid level.

3. Results

A total of 113 (3.7%) nucleotide substitutions were found within the sequenced 513-bp E gene, with a range of 15 (3%) to 24 (4.7%) for viral populations within individuals, harboring more non-synonymous (69, 61.1%) than synonymous substitutions (44, 38.9%) for almost all samples analyzed (Table 1). The mean diversity ranged from 0.19% to 0.32%, with a mean 0.25%. The mean pairwise p-distance ranged from 0.34% to 0.65%, with a mean 0.49%. Except for four transversion nucleotide substitutions, all of the remaining substitutions in the 85 clones studied were transitions, and a high abundance of A→G transitions was observed (data not shown). At the amino acid level, although the consensus sequences of virus populations from individual patients were identical, the mean diversity within individuals ranged from 0.31% to 0.74%, with a mean of 0.47%. The mean pairwise comparison ranged from 0.55% to 1.51%.

* Corresponding author.
E-mail address: tuz1980@126.com (Z. Tu).

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Table 1
Nucleotide and amino acid sequence diversity of the E gene in dengue virus serotype 1-infected patients

Patient[a]	No. of clones	Nucleotide sequence					Amino acid sequence		
		Substitutions[b]		Mean diversity (%)[c]	p-distance (%)[d]		Mean diversity (%)[c]	p-distance (%)[d]	
		Non-silent	Silent		Mean	Range		Mean	Range
3941	15	8	7	0.19	0.34	0–0.79	0.31	0.55	0–1.78
3752	14[e]	10	6	0.22	0.42	0–0.99	0.42	0.75	0–2.96
3987	16[e]	13	9	0.27	0.52	0–1.39	0.48	0.88	0–2.96
4127	17	9	9	0.21	0.42	0–1.19	0.31	0.63	0–2.37
4129	16	15	9	0.29	0.59	0–1.39	0.55	1.09	0–2.37
4130	11	14	4	0.32	0.65	0–1.19	0.74	1.51	0–2.96
Overall	89	69	44	0.25	0.49		0.47	0.90	

[a] All six patients had a classic dengue fever.
[b] The nucleotide substitutions consist of non-synonymous substitutions and synonymous substitutions.
[c] The mean diversity is the number of substitutions divided by the total number of nucleotides or deduced amino acids.
[d] p-distances are calculated by pairwise comparison of nucleotide sequences or amino acid sequences between clones by the MEGA 4 program.
[e] 3752 and 3987 contain a single nucleotide deletion clone at position 1087 and 1008 of the E gene, respectively.

Table 2
Numbers of non-synonymous and synonymous substitutions per site (dN/dS) for a 513-bp fragment of the E gene from different populations

Patient	dN	dS	dN/dS
3941	0.002	0.006	0.431
3752	0.003	0.007	0.463
3987	0.004	0.010	0.408
4127	0.003	0.009	0.306
4129	0.005	0.010	0.503
4130	0.007	0.006	1.080
Mean			0.532

The difference in number of non-synonymous nucleotide substitutions per site (dN) and the difference in number of synonymous nucleotide substitutions per site (dS) for each viral population were calculated with the MEGA 4 program based on the method of Nei and Gojobori. The values of dN/dS were between 0.306 and 0.503 when the patient 4130 population (1.080) was excluded (Table 2). The mean dN/dS ratio was 0.532 for all six patients, indicating that a strong purifying selection has been acting on the population. However, no genome-defective virus (stop codon mutation and indels) was detected in any clone in our study.

4. Discussion

In this study, the mutant spectrum of DENV from individual patients in China was investigated for the first time. The existence of a genetically heterogeneous mixture of variants at the early appearance of symptoms of DENV in mainland China is consistent with the research from neighboring countries.[3–6]

Several studies have found a certain frequency of stop codon mutations in the population – the genome-defective virus.[3–6] Intriguingly, a high frequency of the defective DENV-1 lineage spread in Myanmar from 2001 to 2002, and complementation with functional viruses facilitated their long-term transmission.[4] These findings complicate the characteristics of DENV genetic variation within individuals, and their interactions. However, no genome-defective clone was detected in any clone in our study. Therefore, there are similar but no identical patterns of sequence variation for DENV evolution affecting different human populations.

One major question remains, whether or how to correlate the intrahost diversity to the clinical presentation. Two relatively large-scale studies have provided contradictory results.[5,6] Generally, DENV from the countries of Southeast Asia appear to be more virulent than those initially found in other areas. Although there is a close relationship with Southeast Asian isolates, DENV-1 from South China seldom causes severe dengue infection.[1] Low heterogeneity of DENV-1 and mild dengue fever were found in this study, however much more work should be done before reliable conclusions can be drawn.

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References

1. Wu JY, Lun ZR, James AA, Chen XG. Dengue fever in mainland China. *Am J Trop Med Hyg* 2010;**83**:664–71.
2. Wu W, Bai Z, Zhou H, Tu Z, Fang M, Tang B, et al. Molecular epidemiology of dengue viruses in southern China from 1978 to 2006. *Virol J* 2011;**8**:322.
3. Wang WK, Lin SR, Lee CM, King CC, Chang SC. Dengue type 3 virus in plasma is a population of closely related genomes: quasispecies. *J Virol* 2002;**76**:4662–5.
4. Aaskov J, Buzacott K, Thu HM, Lowry K, Holmes EC. Long-term transmission of defective RNA viruses in humans and Aedes mosquitoes. *Science* 2006;**311**:236–8.
5. Descloux E, Cao-Lormeau VM, Roche C, De Lamballerie X. Dengue 1 diversity and microevolution, French Polynesia 2001–2006: connection with epidemiology and clinics. *PLoS Negl Trop Dis* 2009;**3**:e493.
6. Thai KT, Henn MR, Zody MC, Tricou V, Nguyet NM, Charlebois P, et al. High-resolution analysis of intrahost genetic diversity in dengue virus serotype 1 infection identifies mixed infections. *J Virol* 2012;**86**:835–43.