Phylogenetic characterization of Chikungunya virus isolates from Chennai, Tamil Nadu, India

Chikungunya virus (CHIKV) is a mosquito-transmitted alpha virus belonging to the family Togaviridae[1,2]. It was isolated for the first time in 1952 from a Tanzanian outbreak[3]. It is responsible for an acute infection of abrupt onset, characterized by high fever, arthralgia, myalgia, headache and rash[4]. Poly-arthralgia, the typical clinical sign of the disease is painful. Recently, massive outbreaks have been reported from many islands in the Indian Ocean[5]. Recent phylogenic analysis based on partial sequences of *NS4* and *E1* genes of CHIKV isolates from Andhra Pradesh, Karnataka and Maharashtra showed that they belonged to the African genotype, unlike the earlier isolates which were of Asian genotype[6]. However, the above study did not include any recent isolates from Tamil Nadu. Two Chennai isolates used were isolated in 1964 and 1971. We investigated the genetic phylogeny of the recent CHIKV isolates from Chennai using the partial sequences of *E2* gene of six isolates collected from the city during September–November 2006.

Blood samples were collected from suspected patients of CHIKV infection from the Communicable Diseases Hospital, Chennai in potassium ethylene diamine tetraacetic acid-coated vacutainer tubes (Becton and Dickinson, USA). Plasma was separated and used for RNA isolation using the QIAamp Viral RNA Mini kit (Qiagen, Germany) according to the manufacturer's instructions. cDNA synthesis and PCR were performed in a single tube using the RobusT II single-step RT–PCR kit (Finnzymes, USA). The outer primers that produced a 427 bp product were 5′-TAATGCTGAACTCGGGGACC-3′ and 5′-ACCTGCCACACCCACCAT-CGAC-3′, while the nested primers that amplified a 172 bp internal product were 5′-GATCAGGTTAACCGTGCCGACT-3′ and 5′-CACTGACACAACTACCACA-GTCA-3′ respectively[7]. The single-step RT–PCR cycling conditions were as follows: one cycle at 50°C for 30 min (for cDNA synthesis), one cycle at 94°C for 2 min followed by 30 cycles at 94°C for 30 s, 60°C for 30 s and 72°C for 1 min. This was followed by a final extension at 72°C for 7 min. RT–PCR amplicons and nested-PCR products were run through 2% agarose gels using 100 bp ladder as a DNA size marker. The outer PCR products were purified using the QIAquick PCR purification kit (Qiagen) and sequenced using the Bigdye terminator cycle sequencing ready reaction kit (Applied Biosystems, California, USA) and an automatic sequencer (ABI Prism 3100 Genetic Analyzer, Applied Biosystems). These six sequences have been submitted to the GenBank and have been assigned the accession numbers EF141190–141195.

Using ClustalX, version 1.83, multiple alignments of the obtained nucleotide sequences were performed. The phylogenic status of the CHIKV isolates was assessed using the software[8] MEGA 3.1, Kimura two-parameter distance and neighbour-joining algorithm. The reliability of different phylogenic groupings was evaluated with the bootstrap test (1000 bootstrap replications) available in MEGA. Nucleotide homology percentages were calculated using the commercial sequence analysis software, DNAStar.

The percentage nucleotide homology among all the six CHIKV isolates sequenced varied from 94.8 to 98.5. The percentage nucleotide identity with the Reunion viruses was in the range of 94.8 to 98.8, while the values with the Malaysian genotype viruses were 84.8 to 92.0. The nucleotide homology with the Indian CHIKV, the Nagpur isolate was between 90.8 and 93.9%.

Phylogenic relationship of the six Chennai CHIKV isolates with other sequences available in the GenBank revealed that the Chennai isolates were more closely related to the African genotype virus (Figure 1). These isolates grouped with those seen in outbreaks that occurred in the Indian Ocean islands of Reunion, Mauritius and Seychelles. The CHIKV outbreak in the Reunion Island[9], represents a serious re-emergence of CHIKV

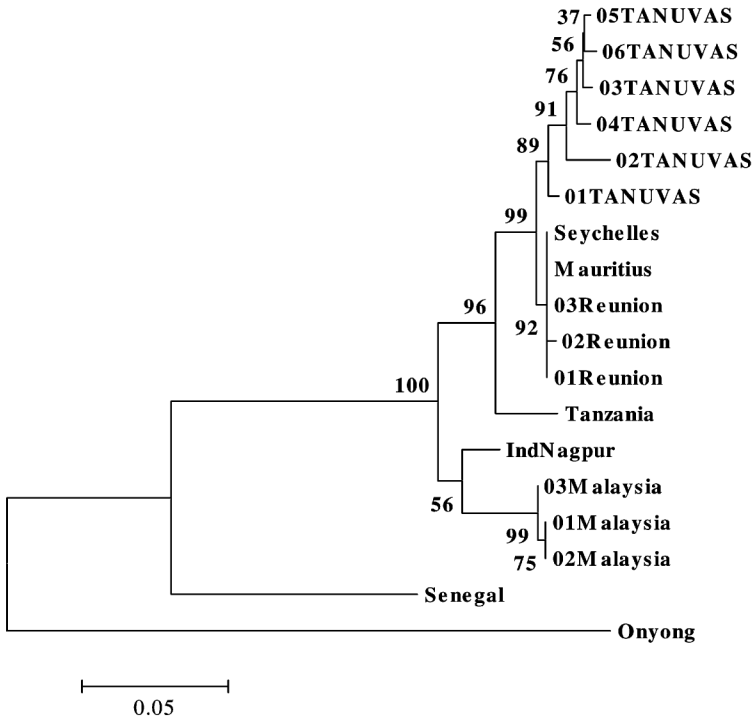

Figure 1. Phylogenic analyses of partial *E2* gene sequences of CHIKV isolates from Chennai, India in relation to other CHIKV sequences. Percentage bootstrap support is indicated by the values at each node. The following sequences were obtained from GenBank: 01 Reunion (AM258992); 02 Reunion (AM258994); 03 Reunion (DQ 443544); Seychelles (AM258991); Tanzania (AF 339485); India Nagpur (AY 424803); 01 Malaysia (AM397003); 02 Malaysia (AM397001); 03 Malaysia (AM 397004), Senegal (AY 726732). Onyong-nyong virus (DQ 383273) was used as an outgroup.

infection during 2005–06. Similar results were reported with isolates from the other three South Indian states by sequencing the *E1* and *NS4* genes[6]. However, the earlier Indian Nagpur isolate of 1965 (sequences submitted in 2003) grouped with the Asian genotype CHIKV (Malaysian lineage). Determining the genotypes of viruses circulating in the different states of India and South East Asia and the conditions favouring such large-scale outbreaks are required to be constantly monitored to control the spread of CHIKV and in the long run to evolve optimum and adequate control strategies.

This study confirms that Chikungunya infection in Tamil Nadu is caused by the African genotype of CHIKV, similar to the ones in the other southern states of Andhra Pradesh, Karnataka and Maharashtra.

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Morphogenetic somatic sieve facilitates 'genomic shock' transmission across cell lineage in plants

The finding by Molinier *et al.*[1] that the capacity of stress-induced increased genomic flexibility is passed onto the successive generations, is a new observation in plant genetics. A parallel to such transgeneration memory could be seen in continual recovery of *in situ* somatic variants that occur in asexual progenies of stressed vegetative propagules[2]. The latter suggests that transgeneration memory of genomic stress owe its genesis to the inbuilt mechanism of 'morphogenetic somatic sieve' in plants.

Incidence of rapid changes in plant genomes to environmental stress has attracted wide attention in recent years, adding to baffling new observations in plant genetics that continue to challenge the current concepts. Particular mention is made of two specific instances: one about heritable phenotypic and genetic alterations that are continuously produced in flax under changed environment[3], and the other about the reversion of mutations in the *Arabidopsis HOTHEAD* gene at an extraordinary high frequency with genome-wide effects[4]. The critical analysis[5] underpinned the significance of consistent reductions in ribosomal gene copy number and widespread insertion events distributed throughout the genome under changed nutritional regimen to bring about sudden but consistent appearance of large element in the genome in flax, and occurrence of extragenomic inheritance in the form of RNA cache of correction templates in *Arabidopsis* respectively, as the possible mechanisms to facilitate rapid changes in plant genomes. However, in the latter this tendency of wild-type reversion of the HOTHEAD mutants has been attributed to their predominant outcrossing nature, since the HOTHEAD plants when grown in isolation exhibit completely stable genetic inheritance[6]. Also, an RNA cache of epigenetic information involving RNA-dependent RNA polymerase to direct paramutation has been experimentally demonstrated in maize to define stability of chromatin states associated with paramutation[7].

Notwithstanding, the experimental demonstration that the plants could inherit even the capacity of stress-induced increased genomic flexibility across generations is quite intriguing[1]. The study pinpoints that in order to cope with the abiotic and biotic stresses, not only do plants activate their own defences, but also manage to pass on a possible protective strategy to their descendents[1,8]. Axiomatically, the ability to increase the frequency of genetic mutation in response to stress has transgeneration memory. Subjecting the *Arabidopsis thaliana* plants to short-wavelength radiation (ultraviolet-C) or flagellin (an elicitor of plant defences), it has been shown that somatic, homologous recombination of a transgenic reporter is increased in the treated population and these increased levels of homologous recombination persist in the subsequent, untreated generations as well[1]. How plants pass down this information is unknown, but Molinier *et al.*[1] opine that the mechanism is 'epigenetic'. Further analysis of RNA directed changes in chromatin structure that cause paramutation lead to speculate that changes in chromatin structure could play a role in passing on the 'memory' of being expressed to environmental stress[9]. The demonstrated genomic change of enhanced homologous recombination is heritable at least to four generations, and could be transmitted through either of the parent regardless of its gender[1]. Concluding their findings, Molinier *et al.*[1] propose that the environmental influences which lead to increased genomic dynamics even in successive,