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Short communication

Molecular epidemiology and evolutionary dynamics of Echovirus 3 serotype

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ABSTRACT

Echovirus 3 (E3) serotype has been related with several neurologic diseases, although it constitutes one of the rarely isolated serotypes, with no report of epidemics in Europe. The aim of the present study was to provide insights into the molecular epidemiology and evolution of this enterovirus serotype, while an E3 strain was isolated from sewage in Greece, four years after the initial isolation of the only reported E3 strain in the same geographical region.

Phylogenetic analysis of the complete VP1 genomic region of that E3 strain and of those available in GenBank suggested three main genogroups that were further subdivided into seven subgenogroups. Further evolutionary analysis suggested that VP1 genomic region of E3 was dominated by purifying selection, as the vast majority of genetic diversity presumably occurred through synonymous nucleotide substitutions and the substitution rate for complete and partial VP1 sequences was calculated to be 8.13×10^{-3} and 7.72×10^{-3} substitutions/site/year respectively. The partial VP1 sequence analysis revealed the composite epidemiology of this serotype, as the strains of the three genogroups presented different epidemiological characteristics.

1. Introduction

Enteroviruses (EVs) comprise a large genus of the *Picornaviridae* family, consisting of different serotypes infecting both humans and animals. E3 serotype has been related with neurologic diseases in humans, such as paralysis, aseptic meningitis and meningoencephalitis (White DO, 1994) as well as with neonatal fulminant hepatitis (Miyata et al., 2014). This serotype was one of the most commonly isolated in the 1970s in USA, but became very rare since then (Khetsuriani et al., 2006). In Europe E3 constitutes one of the most rarely isolated serotypes (Antona et al., 2007; Tan et al., 2011). Recently, it was suggested that this serotype might be re-emerging as a common enterovirus, as it was one of the most commonly isolated serotypes during environmental and clinical surveillance in Georgia and Osaka (Khetsuriani et al., 2010; Sekiguchi et al., 2011) and was also accused for the first reported outbreak in South Africa in 2003 (Yeats et al., 2005).

Human enteroviruses (HEVs) are non-enveloped viruses with a single-stranded, positive sense RNA genome of about 7500 nucleotides, encoding four structural (VP4, VP2, VP3 and VP1) and seven non structural viral proteins ($2A^{pro}$, 2B, 2C, 3A, 3B (VPg), 3C and $3D^{pol}$) (Racaniello, 2007). Enteroviruses, like other RNA viruses, can evolve and adapt rapidly to new environmental conditions. Recombination along with mutations have been recognized as the main mechanisms for enterovirus evolution (Domingo et al., 2008; Savolainen-Kopra and Blomqvist, 2010). Although enterovirus serotypes show over time rapid accumulation of nucleotide substitutions, they have been shown to differ in their epidemiology. Some serotypes like E30, EV71 and E9 (Bailly et al., 2009; McMinn, 2012; McWilliam Leitch et al., 2010) have been associated with large outbreaks occurring periodically showing

Abbreviations: AIC, Akaike information criterion; AFP, acute flaccid paralysis; E, echovirus; EV, enterovirus; ESS, effective sample size; HEV, human enterovirus; MCMC, Markov Chain Monte Carlo; UTR, untranslated region; tMRCA, time to the most recent common ancestor; SLAC, single likelihood ancestor counting; FEL, fixed effects likelihood; FUBAR, fast unbiased bayesian approximation; IFEL, internal fixed effects likelihood; HPD, high-probability distribution; CAV, coxsackievirus A.

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epidemic pattern of circulation while other serotypes, like E6, are characterized by endemic pattern of circulation with no waviness of circulation into a population.

In the present study, we describe an E3 strain (strain EIS6B) isolated in 2009 from the sewage treatment plant of the city of Larissa, Thessaly, Greece, four years after the initial isolation of an environmental E3 strain (LR31G7) in the same geographical region (Kyriakopoulou et al., 2010b), in the absence of other clinical or environmental E3 isolated in Greece. Since few E3 sequences are available in GenBank full genome sequencing of EIS6B strain was performed. In addition, as there has not been conducted any epidemiological and evolutionary analysis of E3 strains, the VP1 sequences of the Greek strains were studied along with those available in GenBank, providing new insights into the epidemiology of this enterovirus serotype.

2. Materials and methods

The enterovirus strain EIS6B was collected from the sewage treatment plant of the city of Larissa, Thessaly, Greece, in June 2009, based on WHO guidelines as described previously (Kyriakopoulou et al., 2010a). The viral RNA was extracted from the Rd cells by the method of Casas et al. (1995) and was reversed transcribed using random primers (Kyriakopoulou et al., 2010a). For the complete genome analysis of strain EIS6B, amplification of overlapping genomic regions was conducted using Paq5000 DNA Polymerase (Agilent Technologies, USA). The details of the primers used for genome amplification are presented in Supplementary Table 1 and the cycling conditions for those designed in the present study are described in Supplementary Table 2. All PCR products were purified with Nucleospin Gel and PCR Clean-up kit (Macherey–Nagel, Germany) and sequenced at CeMIA SA (Larissa, Greece).

Two datasets were determined in order to explore the phylogenetic relationships between E3 strains. The first dataset (45 strains) contained all the sequences for the complete E3 VP1 gene (876 nt) found in GenBank (search 06.05.2014) and the complete VP1 sequence of EIS6B (Supplementary Table 3). The second dataset (111 strains) contained partial E3 VP1 sequences (from 2607 to 2849 nt) derived from GenBank (search 06.05.2014), the overlapping sequences of the complete VP1 dataset and the overlapping sequence of EIS6B (Supplementary Table 3).

Multiple alignments were conducted using ClustalW algorithm implemented in MEGA v6.06 (Tamura et al., 2013). Phylogenetic trees were constructed using the maximum likelihood estimation method and the reliability of the trees was determined by bootstrap analysis with 1000 replicate.

The sequences of both datasets were screened for recombination events that could affect the BEAST analysis by using GARD (Kosakovsky Pond et al., 2006) and SBP methods provided online in Datamonkey website, as well as those provided by the RDP package (Delport et al., 2010). BEAST analyses were carried out based on GTR + G substitution model, according to the Akaike information criterion (AIC) (Posada and Crandall, 2001). Both datasets were analyzed for the estimation of the time to the most recent common ancestor (tMRCA) and the rate of evolution using the Bayesian Markov Chain Monte Carlo (MCMC) method implemented in BEAST package v1.8.0 (Drummond and Rambaut, 2007). BEAST

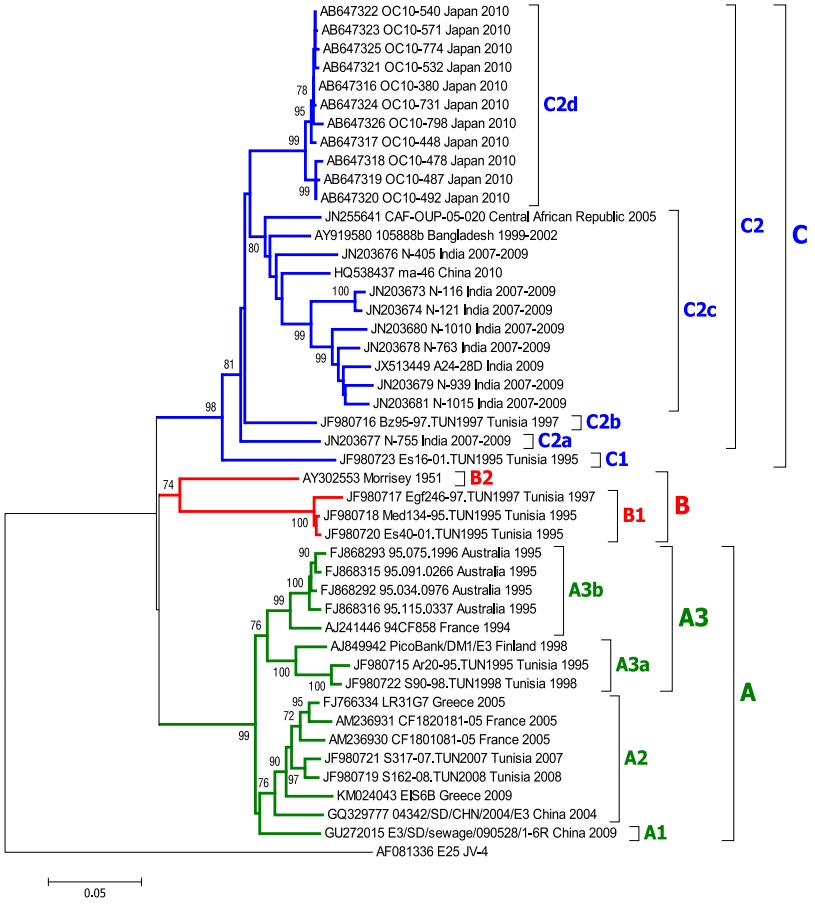

Fig. 1. Phylogenetic tree of the complete VP1 genomic region of EIS6B and all available E3 strains. The tree was constructed using the maximum likelihood estimation method and the bootstrap values were determined for 1000 replicates. Only bootstrap values ⩾70 are shown.