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Baltic lineage of tick-borne encephalitis virus: A putative evolutionary pathway

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ABSTRACT

Tick-borne encephalitis virus (TBEV) is transmitted by ixodid ticks and has three subtypes. The most genetically heterogeneous and widely distributed is the Siberian subtype which is subdivided into two main phylogenetic lineages, *Asian* (TBEV-Sib^{Asia}) and *Baltic* (TBEV-Sib^{Baltic}). According to the hypothesis of quantum evolution of TBEV (Kovalev et al., 2014b), TBEV-Sib^{Asia} originated about 370 years ago in Siberia, but the question concerning the time and place of origin of TBEV-Sib^{Baltic} is still to be solved. In the present paper, the sequences of a gene E fragment of 20 newly obtained TBEV-Sib^{Baltic} strains and 164 sequences of TBEV-Sib^{Baltic} from GenBank were analysed. The clusteron approach applied to TBEV-Sib^{Baltic} allowed the identification of three new clusterons. We revealed three clades of TBEV-Sib^{Baltic}, each characterized by a certain geographical distribution, and estimated their evolutionary ages. The oldest clade was *Balt I*, which presumably originated in North-West Russia and the Baltic countries about 300 years ago as a result of human activity, and then gave rise to the other clades in the Urals and West Siberia. The European subtype of TBEV and TBEV-Sib^{Baltic} may have originated simultaneously from the clusteron-founder 3A of TBEV-Sib^{Asia}, the former through the adaptation of the virus to *Ixodes ricinus*, the latter – to a European subpopulation of *Ixodes persulcatus*. The use of the clusteron approach complemented with the results of phylogenetic analysis, data on the geographical distribution of the virus, the population structure of ticks, and the historical evidence allow us to estimate evolutionary pathways of the subtypes and phylogenetic lineages of TBEV.

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1. Introduction

Tick-borne encephalitis virus (TBEV) is a key member of the TBEV complex within the genus *Flavivirus*, family *Flaviviridae*. The TBEV genome is a positive single-stranded RNA molecule, approximately 11,000 bases in length, which encodes a polyprotein processed into three structural (capsid C, membrane M, envelope E) and seven nonstructural proteins (King et al., 2011). TBEV circulation in natural foci requires ticks as vector and main host for the virus, as well as vertebrate hosts serving as the blood source and making possible co-feeding TBEV transmission between ticks (Labuda et al., 1993). The principal vectors and hosts for TBEV are two species of ixodid ticks, namely *Ixodes ricinus* L. (Europe, North Africa and Middle East) and *Ixodes persulcatus* Schulze (taiga zone from North Japan to the Baltic countries) (Salman, 2012). *I. ricinus* transmits European subtype of TBEV (TBEV-Eu), while *I. persulcatus* serves as a main host for both Siberian (TBEV-Sib) and Far-Eastern

(TBEV-FE) subtypes (Ecker et al., 1999). The TBEV subtypes were revealed based on phylogenetic analysis and are characterized by specific amino acid signatures of the envelope glycoprotein E (Ecker et al., 1999), which is responsible for binding of the virus to the cell surface and plays an important role in the life cycle of flaviviruses (Rice, 1996).

Two main phylogenetic lineages were revealed within TBEV-Sib, Baltic or Eastern-European (TBEV-Sib^{Baltic}), and Asian or Siberian (TBEV-Sib^{Asia}). These lineages can be easily identified by the presence of Asn, characteristic for TBEV-Sib^{Baltic}, or Thr, specific for TBEV-Sib^{Asia}, at the 175 position of the protein E sequence (Golovljova et al., 2008; Karan et al., 2006). Amino acid at the 313 position has been also shown to be of value for their delineation (Golovljova et al., 2008). TBEV-Sib^{Asia} strains are prevalent in Siberia and the Urals, but rarely isolated in the European part of Russia. On the contrary, TBEV-Sib^{Baltic} strains were found only in North-West Russia, Finland and the Baltic countries (Golovljova et al., 2008). Later, however, they were isolated in the Urals and even in West Siberia (Karan et al., 2006; Kovalev et al., 2009). There are two hypotheses about the origin and distribution of TBEV-Sib^{Baltic}. One of them assumes that this phylogenetic lineage originated in North-

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West Russia and the Baltic countries, where it is most prevalent (Golovljova et al., 2008), while the other claims that TBEV-Sib^{Baltic} originated in West Siberia and could have been introduced into North-West Russia as a result of human economic activity during the colonization of Siberia (Kovalev et al., 2009).

In the present paper, genetic variation and evolutionary processes in TBEV-Sib^{Baltic} were studied using the clusteron approach where clusterons are groups of phylogenetically close strains with an identical amino acid sequence of a glycoprotein E fragment and a certain type of geographical distribution (Kovalev and Mukhacheva, 2013). Previously, the clusteron approach has been shown suitable for monitoring virus distribution and spread (Kovalev and Mukhacheva, 2014a), as well as serving as a basis for the hypothesis of quantum evolution of TBEV (Kovalev and Mukhacheva, 2014b). Thus, it has been hypothesized that the evolution of the virus is driven mainly by ticks, with the clusteron structure being a mirror of interspecific (*I. ricinus* and *I. persulcatus*) and intraspecific (subpopulations of *I. persulcatus*) differences between ticks. It has been also suggested that the formation of phylogenetic lineages within a subtype may have been due to the quantum shifts, in particular, for the emergence of TBEV-Sib^{Asia} approximately 370 years ago (Kovalev and Mukhacheva, 2014b). The question concerning the time and place of origin of TBEV-Sib^{Baltic} is, however, still to be solved.

Here, we report an analysis of the genetic diversity of TBEV-Sib^{Baltic} isolates from North-West Russia, the Urals and West Siberia. The clusteron approach and the sequences obtained allowed identification of new clusterons, a phylogenetic analysis of all available TBEV-Sib^{Baltic} strains, studies of their geographical distribution, and, finally, the suggestion of a putative evolutionary pathway of TBEV-Sib^{Baltic}.

2. Materials and methods

Twenty RNA isolates of TBEV-Sib^{Baltic} were isolated from ticks *I. persulcatus*, collected from 2013 to 2015 in Arkhangelsk Region (near Kotlas), Sverdlovsk and Omsk Regions. Detailed information about the place, time and source of isolation is given in Supplement 1. RNA extraction, reverse transcription, PCR and sequencing of glycoprotein E fragment (506 bp) were carried out as described previously (Kovalev and Mukhacheva, 2013). The sequences obtained were deposited in GenBank under the numbers KT749571–KT749577, KT748737–KT748739, KT748742–KT748744, KT748747, KT749597, KT749583, KT749589, KT749621, KC886333, KC886349 (Supplement 1).

Phylogenetic analysis of nucleotide sequences of the gene E fragment (positions 311–762 according to Zausaev AF527415) and deduced amino acid sequences of the glycoprotein E fragment (104–254 aa) was carried out for all isolates obtained in the present paper and 164 sequences of TBEV-Sib^{Baltic} from GenBank. To avoid confusion, hereinafter, isolate and strain will be referred to as a strain. Molecular phylogenetic analysis was inferred by using the Neighbour-joining method based on the Kimura 2-parameter model (Kimura, 1980) using MEGA v. 5.05 (Tamura et al., 2011). Construction of phylogenetic networks for TBEV-Sib was performed using Phylogenetic Network Software v. 4.6.1.0 (fluxus-engineering.com) using an algorithm MJ (Median-joining) (Bandelt et al., 1999). Evolutionary ages of clusterons were estimated based on the previously determined rate of nucleotide substitution, $1.56 \pm 0.29 \times 10^{-4}$ synonymous substitutions per site per year (Kovalev et al., 2009).

The geographical distribution of TBEV strains was analysed based on our own data and GenBank annotation of each sequence used. Information on the geographical distribution of TBEV-Sib clusterons is available as a Supplementary Google Earth map file

Clusterons_TBEV-Sib_2016.kml (Supplement 2). The Google Earth program needs to be installed (<http://www.google.com/earth>).

3. Results

Nucleotide sequences of the gene E fragment were determined for 20 TBEV-Sib^{Baltic} strains, isolated in North-West Russia (n=7), the Urals (n=6) and Omsk Region (n=7). Phylogenetic analysis of nucleotide and deduced amino acid sequences of all available TBEV-Sib^{Baltic} strains (184 in total) allowed identification of three new clusterons, thus showing that clusteron diversity within TBEV-Sib^{Baltic} is significantly greater than it was thought earlier.

3.1. Geographical distribution of TBEV-Sib^{Baltic} and TBEV-Sib^{Asia}

Comparison of prevalence rate of TBEV-Sib^{Baltic} and TBEV-Sib^{Asia} (779 strains) between the European part of Russia, the Urals and Siberia showed an inverse geographical pattern (Table 1). Thus, TBEV-Sib^{Baltic} dominates in the Baltic countries with its prevalence decreasing to single findings in West Siberia. The eastern boundary of TBEV-Sib^{Baltic} is limited by Novosibirsk Region and Altai, where only two strains, 1528 (EF469758) and LEIV-13652A1 (KT224352), were isolated. On the contrary, TBEV-Sib^{Asia} is the most prevalent in East and West Siberia but rarely found in North-West Russia: the strain M2 (JQ693480) was isolated in Moscow Region and two strains, Vologda-157-08 (GQ845434) and Vologda-365-75 (FJ214136), are annotated to be from Vologda region. TBEV-Sib^{Baltic} and TBEV-Sib^{Asia} are hereby “wedged” into each other in a gradual manner thus forming an equivalence zone in the Urals and nearby territories (Table 1).

3.2. Clusteron structure of TBEV-Sib^{Baltic}

Among 184 TBEV-Sib^{Baltic} strains, 145 (78.8%) belonged to clusterons and 39 (21.2%) turned out to be unique. Since the clusteron approach involves only the strains belonging to clusterons (Kovalev and Mukhacheva, 2013), all unique strains were disregarded. Analysis of amino acid sequences of the TBEV-Sib^{Baltic} strains obtained in the present paper, as well as the strains only recently deposited to GenBank by other researchers, allowed identification of three new clusterons, 3W, 3S, and 3Y (Table 2, Fig. 1). The clusteron 3W includes five strains isolated in different years in Estonia (KT748751, KT748752, KT748754, DQ393774, and DQ486861). The clusteron 3S is formed by six strains, isolated in Arkhangelsk Region (near Kotlas) (KT749571–KT749574, KT749576, KT749577). Finally, the clusteron 3Y is represented by only one strain Vologda-365-75 (FJ214136). Formally, this clusteron does not meet the required criterion of a minimum of three strains in a clusteron but its isolation has been justified by the unusual position of the strain Vologda-365-75 in the clusteron structure of TBEV-Sib^{Baltic} as a missing link between the clusteron-founder 3D and its derivative clusterons 3B and 3Q (Table 2, Fig. 1).

3.3. Phylogenetic analysis of the TBEV-Sib^{Baltic}

Phylogenetic analysis of the nucleotide sequences of the gene E fragment of TBEV-Sib^{Baltic} revealed three clades, *Balt^I* (36 strains), *Balt^{II}* (78), and *Balt^{III}* (30). The strain Ekb5263-2011, which was isolated in Sverdlovsk Region and did not belong to any clade, was provisionally regarded here as a member of the clade *Balt^I* (Fig. 2). Each clade includes the strains belonging to the clusteron-founder 3D and a specific set of derivative clusterons: 3G, 3S, 3W, and 3C³ for *Balt^I*; 3B, 3O, 3P, 3Q, and 3Y for *Balt^{II}*; though *Balt^{III}* is formed by only 3D strains (Fig. 2). The clusteron 3C³ from the clade *Balt^I* includes two strains, Ekb4-2009 (JX315986) and Tmn3519-2008 (JX315786), whose amino acid sequences are characterized

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