Nucleotide sequence of the envelope protein of a Turkish isolate of tick-borne encephalitis (TBE) virus is distinct from other viruses of the TBE virus complex

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Turkish tick-borne encephalitis (TTE) virus causes an acute form of meningoencephalomyelitis in sheep in the north-western region of Turkey. The clinical syndrome resembles louping ill (LI) and the viruses responsible for both LI and TTE are members of the tick-borne encephalitis (TBE) complex of the Flaviviridae. The envelope protein gene of TTE virus was reverse-transcribed, amplified, cloned and sequenced. Alignment of the resultant sequence with those from other viruses of the TBE complex reveals that TTE virus is more closely related, at both nucleotide and amino acid levels (84.6% and 96% respectively), to the Central European (CEE) subtype of the TBE virus, usually associated with human disease. The relationship with LI virus is more distant (83% and 93.5% respectively). These studies support the assertion that the ovine encephalomyelitis found in Turkey is caused by a virus that is genetically distinct from known strains of both LI and CEE viruses and from a number of other known viruses of the TBE complex.

In the 1960s an acute form of encephalomyelitis was recognized in sheep in the Gebze area of north-western Turkey (Hartley et al., 1969). The reported clinical signs of Turkish tick-borne encephalitis (TTE) included blindness, tremors, lack of co-ordination, flaccid paralysis, coma and death. All the affected flocks had a moderate infestation of ticks, larvae and nymphs. A similar disease in sheep had previously occurred for at least 15 years in a nearby village. Studies performed confirmed that the causative virus of both foci of infection belonged to the tick-borne encephalitis (TBE) antigenic complex (Hartley et al., 1969).

Louping ill (LI) has been recognized as a disease of sheep, with similar symptoms to TTE, for at least 2 centuries in Scotland (McFadyean, 1900). More recently the disease has spread through many of the upland sheep-raising areas of the British Isles (Reid, 1987). The causal LI virus has been shown also to be a member of the TBE complex (Greig et al., 1931; Andrewes & Pereira, 1978).

Information about the relationship of TTE virus to other viruses of the TBE complex has been obtained from a number of serological studies (Hambleton et al., 1983; Stephenson et al., 1984). These studies confirmed that TTE virus was a member of the TBE complex, but also found that antigenic variation existed between TTE and other TBE viruses.

The purpose of this study was to sequence the envelope (E) protein of TTE virus to elucidate the molecular basis of its antigenic relationship to LI virus and to other viruses in the TBE complex. The E protein is the major surface protein of the virus and is believed to be responsible for a number of important functions. These include eliciting neutralizing antibodies and protective immunity and mediating both receptor binding and low pH-induced membrane fusion of virus membranes with cellular membranes (Guirakhoo et al., 1989; Roehrig et al., 1989; Heinz et al., 1990a).

The virions used in these studies were crudely purified by centrifugation only and the viral RNA was extracted, reverse-transcribed and amplified as described (Whitby et al., 1992). First strand cDNA was synthesized using two antisense primers, 5' CGTGTCCACACGGC-AACCAAC 3' and 5' TTCAGGTGGTACTTGGTTC 3' [complementary to the central European strain, Neudoerfl virus (CEE Neudoerfl), nucleotides 2444 to 2464 and 1785 to 1803 respectively] (Mandl et al., 1988). The E protein gene was then amplified using two sense primers, 5' ATCGAGGCTGGGGCAACCACTGTG 3'
and 5′ TTGGGCACCGGTATTTAC 3′ (nucleotides 1245 to 1268 and 934 to 949 respectively). The two resultant overlapping cDNA fragments, nucleotides 934 to 1803 were compared with the published sequences of other TBE viruses of the TBE complex and the results are shown in Table 1. This analysis revealed that the most similarities in nucleotide and amino acid sequences exist between TTE virus and the two CEE subtypes, CEE Neudoerfl and CEE Kumlinge A52 (Whitby et al., 1993) viruses. Between CEE Neudoerfl virus and TTE virus there were 229 nucleotide changes (84.6 % identical) and 20 amino acid substitutions (96 % identical); for CEE Kumlinge virus as an independent virus of the TBE complex.

The nucleotide sequence and the encoded amino acids of the E protein are shown in Fig. 1. These sequences were compared with the published sequences of other TBE viruses.

Table 1. Percentage homologies of 1488 nucleotides and 496 amino acids of the EPG of TTE virus and other TBE viruses

<table>
<thead>
<tr>
<th>Virus*</th>
<th>Number of nucleotides changes</th>
<th>Identity (%)</th>
<th>Number of amino acids changes</th>
<th>Identity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEE Neudoerfl</td>
<td>229</td>
<td>84.6</td>
<td>20</td>
<td>96.0</td>
</tr>
<tr>
<td>CEE Kumlinge</td>
<td>231</td>
<td>84.5</td>
<td>19</td>
<td>96.2</td>
</tr>
<tr>
<td>RSSE Sofjin</td>
<td>262</td>
<td>82.4</td>
<td>35</td>
<td>92.9</td>
</tr>
<tr>
<td>RSSE 205</td>
<td>266</td>
<td>82.1</td>
<td>34</td>
<td>93.1</td>
</tr>
<tr>
<td>LI</td>
<td>253</td>
<td>83.0</td>
<td>32</td>
<td>93.5</td>
</tr>
<tr>
<td>Negishi</td>
<td>257</td>
<td>82.7</td>
<td>34</td>
<td>93.1</td>
</tr>
<tr>
<td>LGT TP21</td>
<td>364</td>
<td>75.5</td>
<td>68</td>
<td>86.3</td>
</tr>
</tbody>
</table>

* References for each viral strain are given in the text.

Fig. 1. Nucleotide and amino acid sequences of the TTE virus protein E gene.
Envelope protein amino acid homology

Fig. 2. Schematic representation comparing the sequences of the E proteins of a number of TBE viruses.

in TTE virus (Shiu et al., 1991) confirming its classification as a member of the TBE complex.

It is interesting to note that TTE and LI viruses showed several identical amino acid differences, positions 67, 81, 130, 164, 206 and 335. Further information will be necessary before any of these may be associated with encephalitis of an ovine rather than a human nature.

A schematic representation was prepared of the relationships between TTE virus and other viruses of the TBE complex based on a comparison of the amino acids of their E proteins (Fig. 2). The amino acid similarity coefficients substantiate evidence that TTE virus is most closely related to CEE virus subtypes Kumlinge and Neudoerfl. The relationship of TTE virus with RSSE showed several identical amino acid differences, positions 67, 81, 130, 164, 206 and 335. Further information will be necessary before any of these may be associated with encephalitis of an ovine rather than a human nature.

In conclusion, these studies suggest that TTE virus may be a distinct virus within the TBE complex of viruses, with a closer relationship to those viruses causing human encephalomyelitis, CEE Neudoerfl and CEE Kumlinge, than to LI virus, which most usually causes encephalomyelitis in sheep. Further sequence data will be necessary to elucidate completely the evolutionary relationships of TTE virus to other TBE viruses. Such data would help to clarify the evolutionary origin of this virus and provide useful information concerning the epidemiology and spread of TBE ovine disease. In the absence of this information the evolution of this ovine TBE virus, in its apparently isolated focus, remains intriguing.

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References


MANDL, C. W., HEINZ, F. X. & KUNZ, C. (1988). Sequence of the structural proteins of tick-borne encephalitis virus (western sub-type) and comparative analysis with other flaviviruses. Virology 166, 197–203.


variation among members of the tick-borne encephalitis complex. 
Journal of General Virology 65, 81–89.

Nucleotide sequence of the envelope glycoprotein of Negishi virus 
shows a very close homology to louping ill virus. Virology 190, 
515–521.

reverse transcription and amplification of flaviviral RNA following 
low level methyl mercury hydroxide denaturation. Methods in 
Molecular and Cellular Biology (in press).

sequence of the envelope protein gene of the tick-borne flavivirus, 
Kumlinge A52. Virus Genes (in press).

YAMSHCHIKOV, V. F. & PLETNEV, A. G. (1988). Nucleotide sequence of 
the genome region encoding the structural proteins and the NS1 
protein of the tick borne encephalitis virus. Nucleic Acids Research 
16, 7750.

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