sulted in statistically significantly more toxicity than did fluconazole in the study. Thus, we conclude that the potential benefit of posaconazole as prophylaxis for this group of patients is not without some additional risk.

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Thomas F. Patterson1 and Thomas J. Walsh2

1University of Texas Health Science Center at San Antonio, San Antonio; and 2National Cancer Institute, Bethesda, Maryland

References


Presence of the A226V Mutation in Autochthonous and Imported Italian Chikungunya Virus Strains

To the Editor—Recently, Stan Deresinski [1] discussed the role of the A226V mutation in the increased fitness of chikungunya infection in the vector Aeles albopictus, which, in turn, may expand the potential for chikungunya virus to diffuse to the Americas and Europe as a result of the widespread distribution of this vector [2–5].

The recent outbreak in central Italy drew attention to the question of why no other outbreaks had occurred earlier in other regions of Italy or Europe. Recently, Rezza et al. [6] and Charrel et al. [7] listed as major outbreak determinants the concentration of competent vectors at the time of arrival of the index case and the temporal overlapping of arthropod activity (i.e., seasonal synchronicity).

Because the strains that were isolated during the Italian outbreak displayed the A226V mutation, we analyzed the presence of the E1 molecular signatures associated with the Indian Ocean outbreak of chikungunya infection in viral isolates from the 2007 local outbreak and from cases previously imported to Italy. Five isolates from imported cases, 3 of which occurred in individuals returning from Mauritius in 2006 (strains ITA2_BMI_06, ITA1_TAM_06, and ITA3_CGO_06, obtained during the period February–April 2006) and 2 of which occurred in individuals returning from India in 2006 and 2007 (strains ITA4_MRA_06, obtained in September 2006, and strain ITA5_JEM_07, obtained in July 2007), and 2 isolates from autochthonous cases that occurred during the 2007 Italian outbreak (strains ITA7_BIO_07 and ITA8_VEN_07, obtained in September 2007) were included in the sequence analysis, together with an isolate obtained from a mosquito during the Italian outbreak (available from GenBank).

All 7 isolates from humans and the isolate from the mosquito carried the Indian Ocean outbreak signature mutations M269V and D284E. The A226V mutation was present in each of the isolates imported from Mauritius, in the isolates obtained from the autochthonous cases from the Italian outbreak, in the isolates obtained from the mosquito, and in the isolate imported from India in 2007, but it was absent from the isolate obtained in the case imported from India in 2006.

Our findings indicate that, during 2006 and 2007, multiple strains were imported to Italy from the Indian Ocean region. Both the imported and autochthonous strains isolated in Italy displayed the M269V and D284E mutations. A226V was present in all imported and autochthonous strains, with the exception of the isolate imported from India in 2006. This is in agreement with published data [8] and with available GenBank sequence data, indicating that the virus strains circulating in India in 2006 lacked this mutation. On the contrary, according to GenBank sequence data, it appears that this mutation is absent from the most recent isolates obtained in Singapore but is present in each of the isolates obtained during the 2007 outbreak in India (table 1).

These data support the view that the E1 sequence of strains from India changed over time, acquiring the mutation associated with enhanced fitness in A. albopictus after 2006. Fixation of A226V may be a common evolution pathway in explosive outbreaks. Of note, the outbreak in Singapore, where the virus displayed the wild-type amino acid at position 226, was rapidly controlled. Additional studies are needed to appreciate the significance of
this mutation for the interaction of the virus with the vector and with humans, as well as to predict the evolution of outbreaks both at the site of origin and in those countries, such as Italy, to which the virus can be imported.

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Licia Bordi,1 Fabrizio Carletti,1 Concetta Castilletti,1 Roberta Chiappini,1 Vittorio Sambri,1 Francesca Cavrini,1 Giuseppe Ippolito,2 Antonio Di Caro,1 and Maria R. Capobianchi1

1Laboratory of Virology and 2Department of Epidemiology, National Institute for Infectious Diseases “L. Spallanzani”, Rome, and 3Laboratory of Microbiology, Alma Mater Studium, Bologna University, Bologna, Italy

References


Reprints or correspondence: Dr. Maria Rosaria Capobianchi, Laboratory of Virology, Padiglione Baglivi, National Institute for Infectious Diseases, INMI “L. Spallanzani”, Via Portuense 292, 00149 Rome, Italy (capobianchi@inmi.it).

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Table 1. Molecular signatures of the Indian Ocean subgroup in the E1 sequence of 7 chikungunya virus isolates.

<table>
<thead>
<tr>
<th>Chikungunya virus strain (GenBank accession number)</th>
<th>Time of isolation</th>
<th>Provenance</th>
<th>E1 mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>S27a (AF 369024)</td>
<td>1953</td>
<td>Tanzania</td>
<td>A226V M269V D284E</td>
</tr>
<tr>
<td>LR2006_OPY1 (DD443544)</td>
<td>2006</td>
<td>Réunion</td>
<td>Yes Yes Yes</td>
</tr>
<tr>
<td>ITA1_TAM_E1b (EU188924)</td>
<td>February 2006</td>
<td>Mauritius</td>
<td>Yes Yes Yes</td>
</tr>
<tr>
<td>ITA2_BMI_06b (EU190879)</td>
<td>March 2006</td>
<td>Mauritius</td>
<td>Yes Yes Yes</td>
</tr>
<tr>
<td>ITA3_CGO_06b (EU190881)</td>
<td>April 2006</td>
<td>Mauritius</td>
<td>Yes Yes Yes</td>
</tr>
<tr>
<td>IND-06-AP3 (EF027134)</td>
<td>2006</td>
<td>India</td>
<td>No Yes Yes</td>
</tr>
<tr>
<td>MIV-69 (EU350535)</td>
<td>2007</td>
<td>India</td>
<td>Yes Yes Yes</td>
</tr>
<tr>
<td>ITA4_MRA_06b (EU190884)</td>
<td>September 2006</td>
<td>India</td>
<td>Yes Yes Yes</td>
</tr>
<tr>
<td>ITA5_JEM_06 (EU272130)</td>
<td>July 2007</td>
<td>India</td>
<td>Yes Yes Yes</td>
</tr>
<tr>
<td>ITA7_BIO_07 (EU272132)</td>
<td>September 2007</td>
<td>Italy</td>
<td>Yes Yes Yes</td>
</tr>
<tr>
<td>ITA8_VEN_07b (EU272133)</td>
<td>September 2007</td>
<td>Italy</td>
<td>Yes Yes Yes</td>
</tr>
<tr>
<td>ITA07_RA1 (EU244823)</td>
<td>September 2007</td>
<td>Italy (A. albopictus)</td>
<td>Yes Yes Yes</td>
</tr>
<tr>
<td>SG_EHiss622 (EU441883)</td>
<td>2008</td>
<td>Singapore</td>
<td>No Yes Yes</td>
</tr>
</tbody>
</table>

NOTE. Data are shown for 3 chikungunya virus isolates deriving from patients returning to Italy from Mauritius, 2 deriving from patients returning from India, and 2 deriving from patients involved in the 2007 Italian outbreak. The S27 prototype strain, strains from Réunion, the Indian subcontinent, and Singapore, and an isolate obtained from a mosquito during the Italian outbreak (which had an E1 sequence that was available in GenBank) are included for comparison.

a Prototype strain.
b Strains from Italian cases, either imported or autochthonous, analyzed at the National Institute for Infectious Diseases in Rome, Italy.
c Strain from an isolate obtained during the Italian outbreak from Aedes albopictus (Bonilauri P, personal communication); the sequence was available in GenBank.

Novel Risk Factors for Clostridium difficile–Associated Disease in a Setting of Endemicity?

To the Editor—In a retrospective cohort study involving 382 hospital admissions of patients with Clostridium difficile–associated disease (CDAD) and 35,704 hospital admissions of patients without CDAD, Dubberke et al. [1] identified several risk factors for the development of CDAD in a setting of endemicity. Novel risk factors included previous use of intravenous vancomycin and “CDAD pressure” (defined...