Virulence-associated Genetic Changes in Enterovirus D68 Isolates from the 2014 Outbreak

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Introduction

- The 2014 Enterovirus D68 (EV-D68) outbreak in the US reported 1,153 confirmed cases including 14 deaths1. 
- Between 2012 and 2014, several clusters of Acute Flaccid Myelitis (AFM) cases in Colorado2,3, California4,4, France5, and Norway6 were determined to be EV-D68 positive.
- We explored the potential link between genetic changes in the recent EV-D68 isolates and disease severity in the 2014 EV-D68 outbreak using comparative genomics approaches and the Virus Pathogen Resource7 (VIPR, www.viprbrc.org).

EV-D68 Lineage Relationships

Figure 1. Lineage relationships of EV-D68 isolates inferred from VP1 phylogeny. (A) A RAxML phylogenetic tree of all available full-length VP1 nucleotide sequences as of Feb. 9, 2016 in VIPR. 2013-2014 EV-D68 isolates distribute among three clades, suggesting that three separate lineages of EV-D68 were co-circulating. (B) A close-up of subclade B1. Of note, all isolates associated with AFM belong to this subclade.

Neurovirulence-Phylogeny Correlation

Table 1. Neurovirulence-phylogeny association analysis using the BaTS program8 shows that AI, PS, MC (neurovirulent), and MC (non-neurovirulent) statistics were all significant, suggesting that the neurovirulent phenotype is genetically correlated with phylogenetic structure.

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Observed Mean</th>
<th>95% CI</th>
<th>Null Mean</th>
<th>Null 95% CI</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>AI</td>
<td>1.6079</td>
<td>1.1240 - 2.0944</td>
<td>2.5287</td>
<td>2.0605 - 2.9796</td>
<td>0.0002</td>
</tr>
<tr>
<td>PS</td>
<td>10.1675</td>
<td>10.0000 - 11.0000</td>
<td>11.3886</td>
<td>11.3257 - 12.0000</td>
<td>0.0010</td>
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<tr>
<td>MC (neurovirulent)</td>
<td>2.0938</td>
<td>2.0000 - 2.0000</td>
<td>1.1985</td>
<td>1.0000 - 1.8685</td>
<td>0.0002</td>
</tr>
<tr>
<td>MC (non-neurovirulent)</td>
<td>82.7851</td>
<td>81.0000 - 3.0000</td>
<td>49.8717</td>
<td>27.6994 - 80.7979</td>
<td>0.0032</td>
</tr>
</tbody>
</table>

*AI: association index; PS: parsimony score; MC: monophyletic clade

Unique Substitutions in Subclade B1

Table 2. Twenty-one unique substitutions were identified in isolates from the EV-D68 B1 subclade in comparison with non-B1 EV-D68 using the Meta-CATS algorithm9 and subsequent sensitivity and specificity filtering. P-values were corrected by a statistical test that specifically corrects for evolutionary correlation among isolates. Twelve of new substitutions are found in equivalent positions of other enteroviruses known to cause neurological symptoms, including EV-D70, poliovirus (PV), and EV-A71 viruses.

<table>
<thead>
<tr>
<th>Position</th>
<th>Substitution</th>
<th>AI</th>
<th>PS</th>
<th>Null Mean</th>
<th>Null 95% CI</th>
<th>Significance</th>
</tr>
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<tbody>
<tr>
<td>5'UTR/280C</td>
<td>2C/273G</td>
<td>0.0001</td>
<td>0.0001</td>
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<tr>
<td>VP2/222T</td>
<td>640</td>
<td>0.0001</td>
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<tr>
<td>VP3/24A</td>
<td>5'UTR/262C</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
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<tr>
<td>VP1/290S</td>
<td>5'UTR/339T</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

*EV-D68 numbering is based on US/CO/13_60.
*PV numbering is based on PV-1 Mahoney NC_002058.
# Hypervariable region
* Gap
- Not available

Virulence and Clinical Symptom Distribution

Figure 3. Hypothetical model of enterovirus virulence and clinical symptom distribution. Distributions of disease severity caused by B1, non-B1, and PV isolates are represented by hypothetical curves in red, blue, and green, respectively. For a given isolate lineage, disease severity would be influenced by the genetic background and co-morbidities of the infected individual. Symptomatic threshold (72%) and paralytic threshold (1%) represented by the black dashed lines are based on the clinical features of PV infections10.

Conclusion

- Three distinct clades of EV-D68 were co-circulating during the 2014 outbreak with AFM-associated isolates belonging exclusively to a single phylogenetic subclade B1.
- The B1 subclade has 21 unique substitutions in comparison with other EV-D68 lineages.
- Twelve of these substitutions are observed at the equivalent positions in poliovirus, EV-D70, and/or EV-A71.

Future Plan

We are constructing targeted substitutions in EV-D68 genes based on the substitutions identified above in various reporter constructs to test their effects in a variety of different cell culture model systems.

References


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