Comparative Genomic Analysis of D68 Isolates from the 2014 Enterovirus Outbreak

Yun Zhang
J. Craig Venter Institute
July 11, 2015
Enterovirus D68 Outbreak

• Mild to severe respiratory illnesses

  o 1,153 confirmed cases including 14 deaths in the US, likely millions of mild EV-D68 infections (CDC, 2015)

• EV-D68+ Acute Flaccid Paralysis (AFP) cases
  o Colorado (Aug. 2014): 5 out of 11 AFP cases (Messacar, 2015)
  o California (June 2012 – June 2014): 7 out of 16 AFP cases (Greninger, 2015)
  o France (Lang, 2014): 1 case
  o Norway (Bragstad, 2014): 1 case
novel D68 lineage $\rightarrow$ increased disease severity severe neurologic symptoms
**Picornaviridae**

Non-enveloped, +ssRNA

<table>
<thead>
<tr>
<th>Family: Picornaviridae</th>
<th>(26 Genera - 2316 complete genomes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genus: Aphthovirus</td>
<td>(5 Species - 481 complete genomes)</td>
</tr>
<tr>
<td>Genus: Aquamavirus</td>
<td>(1 Species - 2 complete genomes)</td>
</tr>
<tr>
<td>Genus: Avihepatovirus</td>
<td>(1 Species - 114 complete genomes)</td>
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<td>Genus: Avisivirus</td>
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<tr>
<td>Genus: Cardiovirus</td>
<td>(2 Species - 76 complete genomes)</td>
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<td>Genus: Cosavirus</td>
<td>(2 Species - 6 complete genomes)</td>
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<tr>
<td>Genus: Dicipivirus</td>
<td>(1 Species - 0 complete genomes)</td>
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<tr>
<td>Genus: Enterovirus</td>
<td>(15 Species - 1341 complete genomes)</td>
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- **Enterovirus A**: A71
- **Enterovirus C**: poliovirus
- **Enterovirus D**: D68, D70

| Enterovirus A         | (13139 strains - 565 complete genomes) |
| Enterovirus B         | (13074 strains - 182 complete genomes)  |
| Enterovirus C         | (6124 strains - 397 complete genomes)   |
| Enterovirus D         | (746 strains - 13 complete genomes)     |
| Enterovirus E         | (17 strains - 5 complete genomes)       |
| Enterovirus F         | (68 strains - 12 complete genomes)      |
| Enterovirus G         | (144 strains - 7 complete genomes)      |
| Enterovirus H         | (14 strains - 1 complete genome)        |
| Enterovirus J         | (5 strains - 2 complete genomes)        |
| Rhinovirus A          | (2515 strains - 100 complete genomes)   |
| Rhinovirus B          | (613 strains - 33 complete genomes)     |
| Rhinovirus C          | (1755 strains - 19 complete genomes)    |
Sequence Analysis Workflow

1. Search for sequences
2. Construct phylogenetic tree
3. Compare EV-D68 AFP cluster with other clusters
4. Compare EV-D68 AFP cluster with related enteroviruses
5. Infer structural and functional implications of substitutions
Unique Substitutions in B.1.2

B.1.2 vs. non-B.1.2

Sensitivity = \( \frac{TP}{TP + FN} \)

Specificity = \( \frac{TN}{TN + FP} \)

B.1.2 vs. PV, D70, A71

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<th>B.1.2 Representative NT/AA</th>
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<td>31 R</td>
<td>10 K, 1 R</td>
<td>1.43E-08</td>
<td>K</td>
<td>91%</td>
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B.1.2 Substitutions in Nonstructural Proteins

3C cleavage site

2B 2C

2C/1G

catalytic domain

3D

3D/274K
B.1.2 Substitutions in Capsid Proteins

4WM7: EV-D68 Fermon in complex with pleconaril

- VP1
- VP2
- VP3
- VP4
- VP1 pocket
- pleconaril
- B.1.2 substitutions
B.1.2 Substitutions in 5’UTR

Internal Ribosome Entry Site (IRES)

5'UTR structure of poliovirus (Stewart SR and Semler BL, 1997)
Summary

• AFP-associated isolates during the 2014 outbreak belong to cluster B.1.2.

• Cluster B.1.2 has at least 28 unique substitutions.

• Fourteen B.1.2 unique substitutions are observed at the equivalent positions of poliovirus, EV-D70, and/or EV-A71 isolates.
Acknowledgement

Richard Scheuermann

Guangyu Sun

Chris Larson

Zhiping Gu

US NIH/NIAID

Hongtao Zhao

No. HHSN272201400028C

Sherry He

Ed Klem