Genetic Changes Associated with Paralysis 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, California4,5, France6, and Norway7 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 Resource (VPR) (www.viprbrc.org).

EV-D68 Lineage Relationships

Figure 1. Lineage relationships of EV-D68 isolates inferred by phylogenetic analysis of VP1 nucleotide sequences. 2013-2014 EV-D68 isolates distribute among three clades, suggesting that three separate lineages of EV-D68 were co-circulating. Of note, all isolates associated with AFM belong to the single B.1.2 cluster.

Unique Substitutions in Cluster B.1.2

Table 1. Twenty-eight unique substitutions were identified in isolates from the EV-D68 B.1.2 cluster in comparison with non-B.1.2 EV-D68 using the Meta-CATS algorithm8. Fourteen 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.

B.1.2 Substitutions in Capsid Proteins

Figure 3. Locations of B.1.2-unique substitutions in capsid protein structure (PDB ID: 4WM7). A. Surface filling view of EV-D68 capsid proteins. B. Secondary structure view of EV-D68 capsid proteins. VP1/280 and VP1/290 are located on the surface and close to putative receptor binding site8. VP1/194 and VP3/24 are located within the VP1 pocket near the binding site for the anti-viral drug pleconaril. The VP1 pocket is thought to play an important role in the stabilization of virion particles and to facilitate virion uncoating following virus entry.

B.1.2 Substitutions in 5’ UTR

Figure 4. Locations of B.1.2-unique substitutions in 5’UTR structure of poliovirus. Mapping of EV-D68 B.1.2-unique substitutions to this secondary structure representation shows that six substitutions (127U, 188A, 262C, 280C, 339T and 389G) are positioned in the IRES element: 127U and 188A (corresponding to 123 and 185 on PV-1 Mahoney, respectively) are positioned one nucleotide upstream of the stem loop structures II and III with the substitutions predicted to add an additional base pair at the base of these stems; 262C, 280C, 339T, and 389G are positioned within stem loop structure IV.

Conclusion

• Three distinct clades of EV-D68 were co-circulating during the 2014 outbreak with AFM-associated isolates belonging exclusively to a single phylogenetic cluster B.1.2.
• The B.1.2 cluster has 28 unique substitutions in comparison with other EV-D68 lineages.
• Fourteen 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.

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References