Virology 526 (2019) 72–80

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/virology

Large-scale genomic analysis reveals recurrent patterns of intertypic recombination in human enteroviruses

Marios Nikolaidis[a], Kalliopi Mimouli[a], Zaharoula Kyriakopoulou[b,c], Michail Tsimpidis[a], Dimitris Tsakogiannis[b], Panayotis Markoulatos[b], Grigoris D. Amoutzias[a,*]

[a] Bioinformatics Laboratory, Department of Biochemistry and Biotechnology, University of Thessaly, Larisa 41500, Greece
[b] Molecular Virology Laboratory, Department of Biochemistry and Biotechnology, University of Thessaly, Larisa 41500, Greece
[c] Microbiology Laboratory, Department of Medical Laboratories, University of Applied Sciences of Thessaly, Larisa 41110, Greece

ARTICLE INFO

Keywords:
Enterovirus
Rhinovirus
Recombination
Hotspot
Whole genome analysis
Evolution
Poliovirus
E30
E6
EV71
CVA6

ABSTRACT

Recombination is a driving force for the emergence, evolution and virulence/epidemics of viruses, comprising the *Enterovirus* genus of the *Picornaviridae* family, important for human and animal health. By analyzing 2949 complete genomes/coding sequences, we provide a thorough and up-to-date overview of the genome-wide patterns and hotspots of intertypic recombination between the genogroups of this genus. Two prominent recombination hotspots are identified/verified, at the 5′UTR-capsid region junction, and at the beginning of the P2 region. In general, P2 was enriched in recombination events. Key phylogenetic groups implicated in recombination events are E71 and CVA6 in *Enterovirus A* species, E30 and E6 in *Enterovirus B* species, polioviruses 1 and 2 in *Enterovirus C* species. In addition, many events involve recombination partners that have not been sequenced yet, thus strongly suggesting a large environmental reservoir of genetic variation with a high potential for the emergence of new modified pathogens by recombination.

1. Introduction

Viruses of the *Enterovirus* genus of the *Picornaviridae* family of positive strand RNA viruses comprise many species important for human and animal health (Whitton et al., 2005). In particular, they cause severe infections such as poliomyelitis, aseptic meningitis, hand-foot and mouth disease, myocarditis and type I diabetes among others (Akerblom et al., 2002; Pallansch and Roos, 2007; Stanway, 2013). They also cause many subclinical infections with common-cold like symptoms (Muehlenbachs et al., 2015), thus, their impact upon society and economy can be very significant (Gan et al., 2015; Khetsuriani et al., 2003; Liu et al., 2016; Parasuraman et al., 2001).

The emergence, evolution, and virulence/epidemics of RNA viruses (Malim and Emerman, 2001; Nora et al., 2007; Simon-Loriere and Holmes, 2011; Xiao et al., 2016), including enteroviruses is significantly fueled by recombination. This process creates chimeric molecules from parental genomes of different phylogenetic origins (Combelas et al., 2011; Krogstad et al., 2008; Kyriakopoulou et al., 2014; Lin et al., 2015; Lukashev, 2005; Lukashev et al., 2014, 2005; McIntyre et al., 2013; Oberste et al., 2004; Oprisan et al., 2002; Pliaka et al., 2012; Savolainen-Kopra and Blomqvist, 2010; Simmonds and Welch, 2006). The underlying molecular mechanisms involve template switching (Arnold and Cameron, 1999; Kirkegaard and Baltimore, 1986) by the RNA polymerase via an intermediate step of duplicated segments (Lowry et al., 2014) or the replication-independent joining of RNA molecules (Gmyl et al., 1999). Recombination may act against Muller's Ratchet (Felsenstein, 1974; Muller, 1964) and as a counterbalancing force against high mutation rates (Xiao et al., 2016), by eradicating deleterious mutations. It may also lead to the combination of advantageous properties from various genomes into a new one, or to the emergence of drug resistance, or even evasion from the immune system. A very practical implication involves the development of live vaccines, where previously attenuated vaccine strains recombine with other circulating strains. This may lead to the emergence of new genomes that are functional and capable of causing new epidemics, as is the case with vaccine derived polioviruses (Burns et al., 2013; Guillot et al., 2000; Karakasiliotis et al., 2005; Kew et al., 2002; Paximadi et al., 2007; Pliaka et al., 2012; Stern et al., 2017).

In many RNA viruses, secondary structure elements have been suggested to facilitate recombination (Bessaud et al., 2016; Dedepsidis et al., 2010; Dutkiewicz et al., 2016; Simon-Loriere et al., 2010). Of note, in single stranded RNA viruses, these secondary structures may

* Corresponding author.
E-mail address: amoutzias@bio.uth.gr (G.D. Amoutzias).

https://doi.org/10.1016/j.virol.2018.10.006
Received 20 April 2018; Received in revised form 4 October 2018; Accepted 4 October 2018
Available online 23 October 2018
0042-6822/

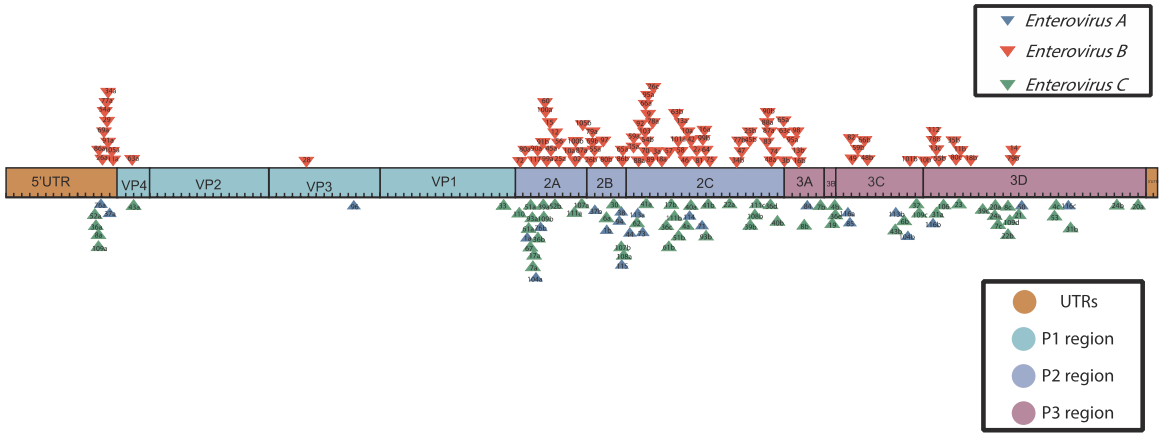

Fig. 1. Recombination events in Enterovirus A, B and C. Triangles depict recombination events. Numbers within triangles correspond to recombination event numbers in supplementary files S19 & S20.

form even when the complementary strand is synthesized. In addition, genomic segments of high sequence similarity may also facilitate recombination between different phylogenetic groups (Baird et al., 2006; Magiorkinis et al., 2003; Simon-Loriere et al., 2010; Zhang and Temin, 1994, 1993). Obviously, the two strains that undergo recombination need to have the same tissue tropism.

The enterovirus genome has a length of ~7.500 nt, with an architecture of a 5′UTR, followed by a single ORF and then by a 3′UTR (Kitamura et al., 1981). The translated ORF is cleaved into four structural (VP1-VP4; P1 region), and seven non-structural protein regions (2A-2C:P2 region and 3A – 3D:P3 region) (Wimmer et al., 1993) (see Fig. 1). Several RNA secondary structures are found across the genome that could potentially facilitate intra-typic or inter-typic recombination (Dedepsidis et al., 2010).

According to the 10th report of the International Committee on Taxonomy of Viruses (ICTV), human enteroviruses belong to the *Enterovirus* genus of the *Picornaviridae* family, and they are classified into seven species, *Enterovirus A* to *D* (EV-A to D), and *Rhinovirus A* to *C* (RV-A to C) (Zell et al., 2017).(www.ictv.global/report/picornaviridae). In general, no exchange of genetic material is observed among strains of different species, with the exception of the highly conserved 5′UTR (Kyriakopoulou et al., 2014). Previous analyses on enteroviruses have shown that there exist two main phylogenetic clusters of 5′UTR regions (Kyriakopoulou et al., 2014; Santti et al., 1999). Cluster I consists mainly of *Enterovirus C* and *D* sequences (with some exceptions of *Enterovirus A*) whereas cluster II consists of *Enterovirus A* and *B* sequences. Exceptions do occur, as demonstrated for EVA90, EVA91 and EVC-109 whose 5′UTRs are found within the other cluster (Kyriakopoulou et al., 2014; T. Smura et al., 2007; T.P. Smura et al., 2007; Yozwiak et al., 2010). Experiments on vaccine derived polioviruses showed that whenever a 5′UTR region was intruding from Cluster II (*Enterovirus A* and *B*), the recombined poliovirus demonstrated decreased viral replication and virulence (Muslin et al., 2015).

The seven species are further divided into 283 distinct phylogenetic types, based on sequence divergence of the VP1 capsid region (Oberste et al., 1999). More specifically, *Enterovirus A, B, C, D* have 25, 63, 23, 5 genotypes, whereas human *Rhinovirus A, B, C* have 80, 32 and 55 genotypes respectively. Intriguingly, there is no clear relationship between genotypes and clinical symptoms, as more than one genotypes may cause the same symptom or the same genotype may be implicated in more than one syndromes.

Many studies over the years have highlighted certain species, genotypes and genomic regions to be frequently implicated in recombination events, as reviewed in (Kyriakopoulou et al., 2014). Furthermore, they have focused on certain genomic regions, but not the whole genome, without exactly locating the point of recombination/s, or they have focused on certain genotypes/ epidemics that manifest symptoms. Thus, it is conceivable that many more recombination events occur in strains with a silent transmission profile. In addition, there exists a significant sampling bias in sequence databases, since genotypes that cause clinical symptoms and/or epidemics are the ones being sequenced. Consequently, there is a need for a large-scale analysis that utilizes and normalizes the wealth of all publicly available genomic data, in order to provide an unbiased as possible global picture of the patterns of intertypic homologous recombination in this virus. The current study attempts to address this issue and analyze 2949 enterovirus/rhinovirus genomes so as to obtain a global picture of which regions of the genome are usually implicated in homologous recombination and whether regions with exceptionally high or low recombination rates exist. This study also maps the network of exchange of genetic material (via recombination) between the various phylogenetic groups. Thus, in the future, a more efficient monitoring that takes into account the co-circulation of various genotypes may predict the emergence of new recombinant forms and epidemics or even lead to the design of better live vaccines that don't recombine, by inserting disrupting mutations in recombination hotspots (Runckel et al., 2013).

2. Materials and methods

Human enterovirus and rhinovirus nucleotide sequences were downloaded from GenBank (Benson et al., 2013), using as keywords the terms "enterovirus complete" and "rhinovirus complete" for each of the corresponding subgroups. Sequences were filtered with a nucleotide length threshold of 6000 bps and each sequence was manually inspected to exclude animal enteroviruses. Thus, 2949 sequences were retained after this step (supplementary FASTA file S1.fa). Also, their corresponding coding sequences (supplementary FASTA file S2.fa) and annotation were retrieved from Genbank.

The prototype strains of the genogroups from human *Enterovirus A, B, C, D* and human *Rhinovirus A, B, C* species and their 274 sequences were retrieved from the Picornaviridae website (http://www.picornaviridae.com/enterovirus/enterovirus.htm) (see supplementary FASTA file S3_reference_seqs.fa). Also, the VP1 capsid sequence that is used for genotyping was extracted for each prototype strain (see supplementary FASTA file S4_reference_VP1_seqs.fa). In order to ensure correct genotype assignment and exclude annotation mistakes in Genbank, a phylogenetic analysis was performed for each of the seven major evolutionary groups (human *Enterovirus A, B, C, D*, human *Rhinovirus A, B, C*), based on the protein sequence of the VP1 region. Multiple sequence alignment was performed with Muscle (Edgar, 2004) (see supplementary files S5_aln_VP1_EVA.fa - S11_aln_VP1_HRVC.fa). A Neighbor Joining tree (BioNJ – Poisson model) with 500 Bootstrap

replicates was created for each subgroup with the Seaview4 software (Gouy et al., 2010) and the 7 trees were visualized with the Treedyn software (Chevenet et al., 2006) (see supplementary files S12.pdf – S18.pdf). Genotype assignment for each sequence (downloaded from Genbank) was based on i) Genbank annotation, ii) best blast (Camacho et al., 2009) hit to the VP1 sequence of reference strains and iii) on the phylogenetic analysis of the VP1 sequence.

Afterwards, redundancy of highly similar sequences was removed by clustering them at a cutoff of 97% sequence identity and keeping one representative sequence per cluster, thus resulting in 684 sequences for the *Enterovirus A, B, C, D* species and 280 sequences for the *Rhinovirus A, B, C* species. The clustering was performed with the UClust algorithm (Edgar, 2010), that we have embedded in the T-RECs software (Tsimpidis et al., 2017). T-RECs is a Microsoft Windows based recombination detection tool that has been developed and published by our group for high-throughput analyses (Tsimpidis et al., 2017). It is based on pairwise alignment of sliding windows and is specifically developed to rapidly and efficiently detect recombination events among different evolutionary groups. It was originally evaluated with a dataset of 555 complete genomes and 2500 sequence fragments from noroviruses (Tsimpidis et al., 2017). Next, the non-redundant enterovirus sequences were analyzed for recombination events with the T-RECs software, by using the FASTA sequence file as both query and database. Identified recombination events (361 for enteroviruses and 72 for rhinoviruses) were further analyzed with similarity plots, again within the T-RECs software and were manually inspected. For the majority of rhinoviruses, only the prototype strain and maybe a few more sequences from each genogroup were available. Thus, we only used the rhinoviruses to detect potential recombinations with enteroviruses. Nevertheless, we did not detect any signs of recombination among rhinoviruses and enteroviruses. Concerning the enteroviruses, we identified 247 sequences with complex recombination events that were not clear and easy to interpret, whereas another 114 sequences had recombination events that could be clearly identified (see Supplementary File S19_Simplots.zip). These 114 sequences with clear recombination events were used in the subsequent analyses. Additionally, for the clear events, annotation of the genomic regions (coordinates of each protein region on the genome) was also integrated in the similarity plots. Based on the similarity plots and the genomic location of each site of recombination, a graphical representation of recombination breakpoints was created for the *Enterovirus A, B, C* species (see Fig. 1).

For each genotype, we collected the number of clearly identified recombination events and also the number of sequences analyzed for that particular genotype (see supplementary file S20). These data were used to create Fig. 3 that shows the absolute number of recombination events per genotype (darker-coloured bars) and the normalized number of recombination events per genotype (lighter-coloured bars), by dividing the absolute number of recombination events with the number of analyzed sequences for that particular genotype. This normalization takes into account the bias that exists in sequence databases towards certain genotypes that cause clinical symptoms/epidemics.

In order to detect the closest intertypic genetic relative of a recombined sequence fragment, each fragment was also analyzed/blasted against the NCBI nr/nt database, using a nucleotide sequence identity threshold of $\geq$ 90%. We also required that the coverage of the query sequence fragment was above 90%. If the top intertypic genetic relative/hit was below that threshold (90% sequence identity), it was considered unknown. As expected, the top blast hit was the sequence itself, but we also allowed for more sequences of the same genotype to be top hits, above the top intertypic genetic relative/hit, as long as their nucleotide identity was not below 97% (due to sequence redundancy). All identified recombination events are stored in supplementary file S20. Based on two thresholds of 90% and 95% sequence identity, three networks of exchange/relatedness of genetic material between different genotypes were created in Cytoscape (Su et al., 2014).

We also analyzed 1426 5′UTR sequences with a BioNJ phylogenetic tree (Jukes-Cantor model, 500 bootstraps), as stated above. More specifically, we originally retrieved 2785 5′UTR sequences (from the downloaded genomes), more than 650 nt long and then removed redundancy by clustering them with 99% nucleotide identity cutoff. The genotyping of each of these 5′UTR sequences was based on the VP1 region of their corresponding genome, as explained in the second paragraph of Materials and Methods, above. The corresponding sequence alignment and BioNJ phylogenetic tree are also available as supplementary files S21 and S22.

3. Results and discussion

3.1. The capsid region exhibits very low recombination rates in EVs

In accordance with the broadly held view in literature that is based on multiple evidence, we also observed the least number of recombination events within the P1 capsid region (see Fig. 1). VP1 is considered the protein region that usually does not recombine, thus genotyping is based on this region. We only observed one recombination event at the end of VP1 in an *Enterovirus C*, that has already been reported in FJ859189 (PV-3) (Zhang et al., 2010). Also, VP3 had two recombination events being detected in *Enterovirus B* CV-B4 (sequence ID: EF371880), already reported by (Bouslama et al., 2007) and *Enterovirus A* CV-A16 (sequence ID: KU163608) respectively (not reported yet). The VP4 region had two recombination events as well detected in sequence KC897073 from E-30, already shown in (Xiao et al., 2014) and JF260925 from CV-A17, already shown in (Bessaud et al., 2011). Other analyses have identified recombination events as well within the capsid region (Blomqvist et al., 2010, 2003; Dedepsidis et al., 2008; Kyriakopoulou et al., 2006). Several reasons have been suggested for the very low recombination frequency within the capsid region. Perhaps the most prominent ones are structural constraints of the virion shell or disruption of receptor binding (Oberste et al., 2004). Another hypothesis is that the high sequence variability of this region does not favor template switching, that needs small segments of high sequence identity between the two parental strains.

3.2. The 5′UTR-VP4 junction is a recombination hotspot

The 5′UTR has an average size of 750 nt and is the most highly conserved region of the enterovirus and rhinovirus genomes. It forms several RNA secondary structure elements with specific functions, such as the cloverleaf, involved in replication and the Internal Ribosome Entry Site (IRES), that is responsible for the translation of the viral genome (Dutkiewicz et al., 2016; Pelletier and Sonenberg, 1988). It is organized in 7 domains and two spacer regions (Muslin et al., 2015). Furthermore, this region interacts with several viral and host proteins and contributes to cell tropism, host range, virulence and pathogenicity (Kawamura et al., 1989; Bradrick et al., 2001; Harvala et al., 2002; Muslin et al., 2015). Nevertheless, recombination at the 5′UTR – VP4 junction should theoretically be non-problematic. In addition, 2D elements of (-) 3′ could enhance recombination at this region. Indeed, past phylogenetic analyses have shown evidence of incongruence between 5′UTR and capsid region trees (Boros et al., 2012; Kyriakopoulou et al., 2014, 2017; Muslin et al., 2015; Santti et al., 1999; Simmonds and Welch, 2006; Yozwiak et al., 2010). The majority of these analyses were focused on specific *Enterovirus* species or genotypes. Also, in vitro recombination experiments of PV genomes with 5′UTRs originating from the four *Enterovirus* species revealed three recombination hotspots (Muslin et al., 2015). One was detected at spacer 1, situated 100 nucleotides downstream from the beginning of the 5′UTR. Another recombination hotspot was identified between domains 5 and 6, around position 600, whereas the third and most prominent one was located at spacer 2, at the end of the 5′UTR. The current large-scale analysis that uses all publicly available complete genomes/CDS (see Fig. 1) strongly supports the previous findings that were based mostly on small/

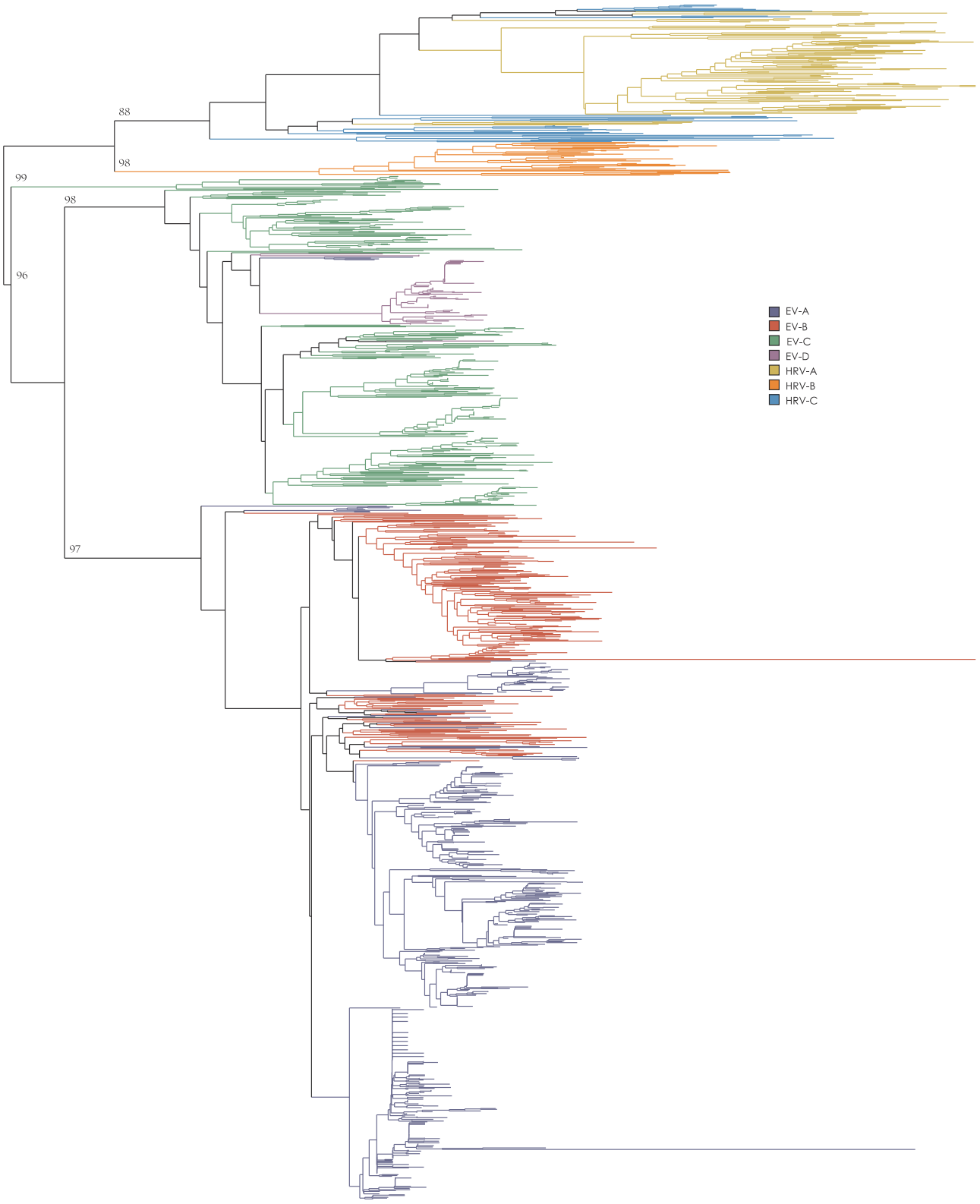

Fig. 2. BioNJ phylogenetic tree of the 5′UTR of human *Enterovirus A-D* and human *Rhinovirus A-C* sequences. Colors correspond to each of the 7 species. Bootstrap values are depicted for each major cluster.

medium-scale analyses of phylogenetic incongruences (focused on specific *Enterovirus* species or genotypes) and partly supports the findings of the in vitro experiments, thus strongly emphasizing the need for an intact 5′UTR. It is conceivable that we did not detect the first recombination hotspot, because of the parameters that we used in our scanning, with a window length of 200 nucleotides long.

Previous analyses on enteroviruses have shown that there exist two main phylogenetic clusters of 5′UTR regions (Kyriakopoulou et al., 2014; Santti et al., 1999). Our analysis further included 5′UTRs from human *Rhinovirus A, B, C* sequences as well and verified previous

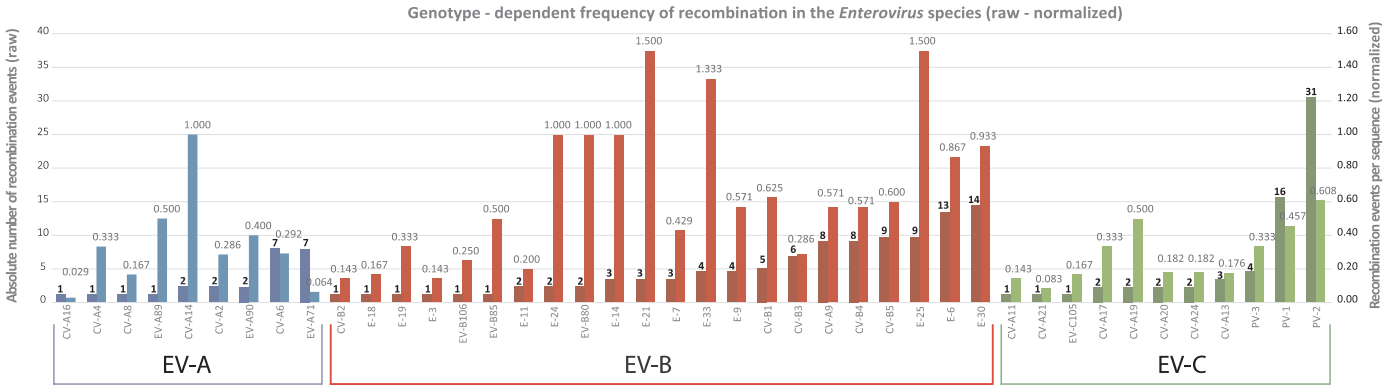

Fig. 3. Recombination events per genotype. Dark coloured bars correspond to absolute number of recombination events per genotype. Some genotypes have many sequences in databases and this bias is not taken into account. On the other hand, these genotypes more frequently cause clinical symptoms/epidemics. Light coloured bars correspond to normalized number of recombination events per genoptype. Here, the absolute number of recombination events is divided by the number of analyzed sequences for that particular genotype.

findings of the two major phylogenetic clusters and their exceptions (see Fig. 2). We observed that human *Rhinovirus B* 5′UTRs formed a distinct monophyletic cluster, whereas *Rhinovirus A* and *C* 5′UTR sequences formed another distinct cluster, but with no clear signs of monophyly. This suggests that *Rhinovirus A* and *C* genomes may exchange 5′UTR regions, but *Rhinovirus B* genomes seem to be isolated. Furthermore, enteroviruses and rhinoviruses did not exchange any sort of genetic material, even though their sequences show clear signs of homology.

3.3. P2 is a recombination hotspot, especially in EV-B & EV-C

Another prominent recombination hotspot that emerges from this large-scale analysis is the P2 region and particularly 2A (see Fig. 1). P2 has been repeatedly reported as a frequent site of recombination in the past literature (Kyriakopoulou et al., 2014; Lukashev et al., 2005; Mirand et al., 2007). More particularly, the 2A region has been reported as a region where recombination breakpoints concentrate, for several different viruses (McWilliam Leitch et al., 2012). Our large-scale analysis that is based on all publicly available complete genomes/CDS unequivocally confirms previous findings of medium/small scale studies (focused on specific *Enterovirus* species or genotypes) and highlights the 2A region as another recombination hotspot in both *Enterovirus B* and *C* sequences. In general, *Enterovirus B* and *C* had a significantly elevated frequency of recombination across the 2A region, but also the 2C region was the focus of many recombination events. On the contrary, *Enterovirus A* strains display a more evenly distributed pattern of recombination points across the whole P2 and P3 regions. A very recent and focused genomic study on Sabin 1, 2, 3 poliovirus recombination patterns from Russia and former Soviet Republics highlighted P2 and especially 2C as a recombination hotspot (Korotkova et al., 2017). P3 also had a very significant number of recombination events. Intriguingly, each of the three Sabin strains had different recombination hotspots. Even though we did not include their sequence data (Korotkova et al., 2017) in our analyses, we also provide a Supplementary figure (S23) that compares the recombination breakpoints detected by that study (Korotkova et al., 2017) (which is geographically and strain focused) with the recombination breakpoints of vaccine derived PVs and other *Enterovirus C* genotypes in our dataset (that is more broad-based). Both our study and that of (Korotkova et al., 2017) identify a significant number of events in P2, but a notable difference is that the latter study finds significantly less events in 2A compared to 2C (see Supplementary Fig. S23).

3.4. Key players in recombination events

Evidence from many past small and medium-scale studies has revealed certain phylogenetic groups as key donors and/or acceptors. In this analysis, we have identified recombined sequence fragments in various genotypes and searched for their closest relatives in the nr/nt NCBI database with Nucleotide Blast. We applied two thresholds of 90% and 95% sequence identity. It is tempting to assume that these identified genotypes (by blast) have acted as (or they are the closest relatives of) donors of genetic material. However, due to double and triple recombinants (that are not uncommon), assigning the source of these regions is difficult since they may represent a common genetic pool from which many different viruses can draw their sequences. We thus prefer the term of “recombination partner”.

More specifically, EV-71 is considered the most prominent of the 25 existing *Enterovirus A* genotypes, due to large epidemics in Southeast Asia. It causes diarrhea, rashes, hand-foot and mouth disease, or even encephalomyelitis (Yogarajah et al., 2017). Another prominent strain is CV-A6, a causal agent of hand foot and mouth disease (Puenpa et al., 2016). Accordingly, many sequenced genomes exist in databases for these prominent genotypes (especially EV-71). Our analysis identified these two genotypes as the most prominent *Enterovirus A* recombination partners (see Figs. 3 and 4). Recombination events were identified mainly at 2A, 2B and 2C regions, with no particular hotspot emerging from this analysis (Fig. 1). However, when normalizing for the number of sequences analyzed, EV-71 appears to have a low propensity for recombination. Indeed, previous analyses have demonstrated this low propensity of EV-71 compared to other *Enterovirus A* and *B* genotypes (Lukashev et al., 2014; McWilliam Leitch et al., 2012). In particular, (Lukashev et al., 2014) estimated that EV-71 is 4–5 times less likely to recombine than CV-A2 and CV-A4, a range of values that we also estimated in our normalized counts (see Fig. 3).

The *Enterovirus B* species contains 63 different genotypes and thus it is not surprising that its strains have been reported to recombine much more frequently than *Enterovirus A* strains (Oberste et al., 2004; Simmonds and Welch, 2006). The most prominent member of this evolutionary group is Echo 30 (E30), the main cause of viral aseptic meningitis (Kyriakopoulou et al., 2017). Previous analyses have highlighted the role of E30 in recombination events, while other genotypes such as E-6 and CV-B4 have been reported as well (Lindberg et al., 2003; Bailly et al., 2011; Kyriakopoulou et al., 2012). Our analysis identified E30, E-6, E-25, CV-B5, CV-B4 and CV-A9 as prominent recombination partners (see Figs. 3 and 5). When normalizing for the number of sequences analyzed, E-30 and E-6 were still displaying a high propensity for recombination and E-25 emerged as one of the most frequently recombining genotypes (see Fig. 3). Of notice, the ratio of

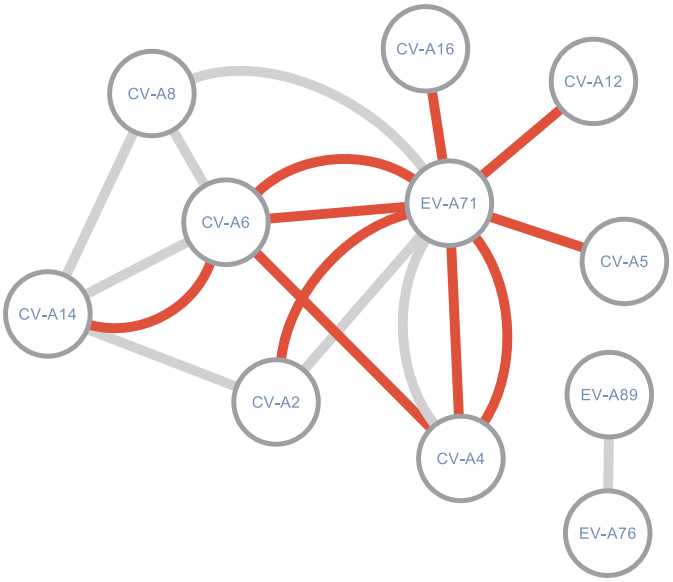

Fig. 4. Network of recombination partners for Enterovirus A genotypes. Edges depict events where the recombined sequence fragment within a certain genotype had ≥ 90% (grey color) or ≥ 95% (red color) identity with another genotype, based on NCBI BLAST. EV-71 and CV-A6 play a central role as recombination partners.

relative recombination frequencies in E6, E30 and E11 is compatible with McWilliam Leitch (2012), which suggested much lower recombination incidence (longer non-recombinant sequence half-life) in E11 compared to E6 and E30. Interestingly, recombination hotspots were identified at the 5′UTR-VP4 junction, 2A, 2B and 2C regions, although recombination events were also detected in the P3 region, albeit with not so high frequency (Fig. 1).

Human *Enterovirus C* species consists of 23 different genotypes, where recombinations are frequent (Combelas et al., 2011; Smura et al., 2014; Bessaud et al., 2016). Based on VP1 phylogeny, *Enterovirus C* is divided in three evolutionary subgroups (see supplementary file S14). The most important one contains polioviruses 1, 2, 3, CV-A13, CV-A17, CV-A20 and EV-C102. Polioviruses are the most prominent members of this species and are the main causative agents of poliomyelitis. However, a successful global vaccination program, mainly with live attenuated/mutated strains, designated as Sabin 1, 2, and 3 has lead to its eradication in many countries. These attenuated vaccine strains have a high propensity to recombine with other *Enterovirus C* genotypes of the same subgroup, such as CV-A13 and CV-A17. These recombinants may form circulating Vaccine Derived PolioViruses (cVDPDs) that cause new outbreaks of poliomyelitis (Joffret et al., 2012; Rakoto-Andrianarivelo et al., 2007), thus jeopardizing vaccination strategies. Our analysis clearly demonstrates that most of the detected recombination events occur among the three polioviruses, where PV2 emerges as the most frequent recombination partner, followed by PV1 (see Figs. 3 and 6). This observation holds even when normalizing for the number of sequences analyzed (see Fig. 3). In another study of Sabin poliovirus recombinants isolated from Russia and former Soviet Republics, the majority of recombinants were PV2 and PV3 (Korotkova et al., 2017). The data in our study show that PV2 also shares genetic material with CV-A13 and CV-A17, that belong to the same evolutionary subgroup with

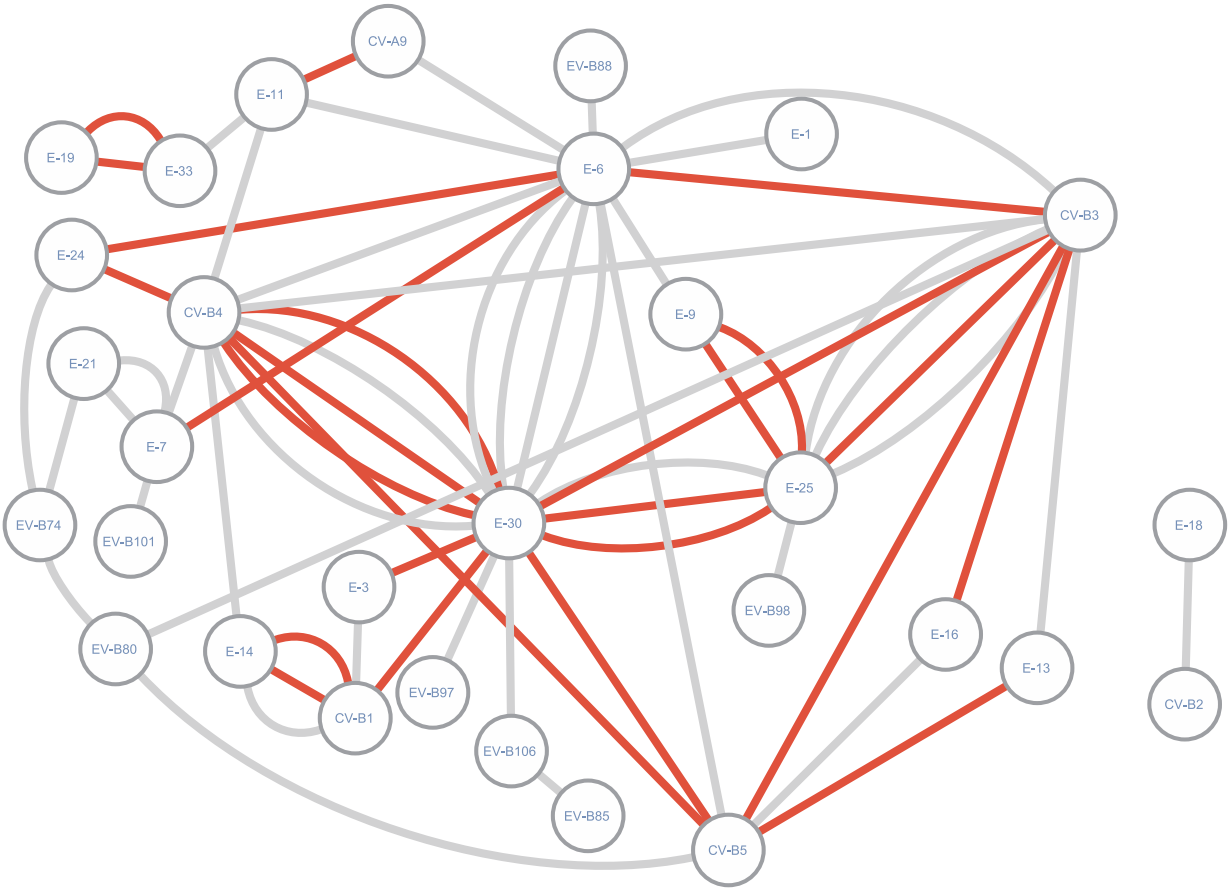

Fig. 5. Network of recombination partners for Enterovirus B genotypes. Edges depict events where the recombined sequence fragment within a certain genotype had ≥ 90% (grey color) or ≥ 95% (red color) identity with another genotype, based on NCBI BLAST. E30, E-6, E-25, CV-B5, CV-B4 and CV-A9 emerge as prominent recombination partners.

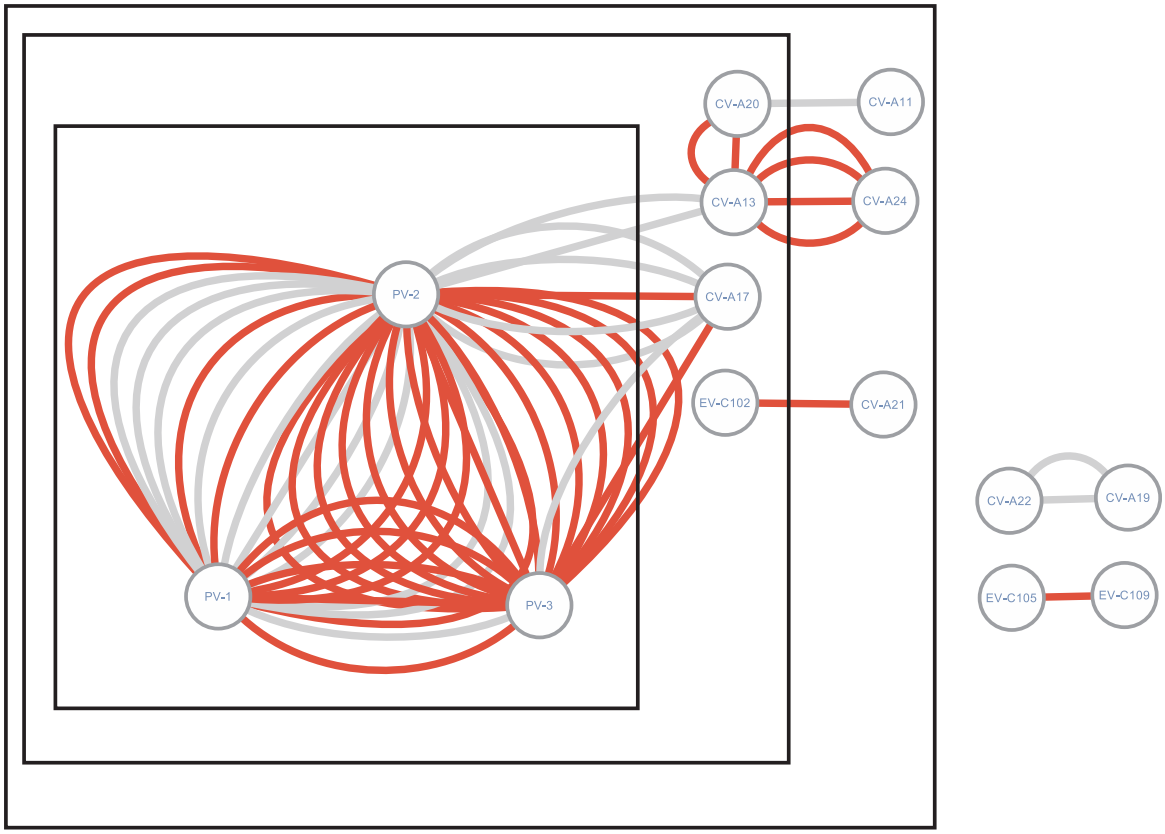

Fig. 6. Network of recombination partners for Enterovirus C genotypes. Edges depict events where the recombined sequence fragment within a certain genotype had ≥ 90% (grey color) or ≥ 95% (red color) identity with another genotype, based on NCBI BLAST. Boxes group genotypes according to the phylogenetic relatedness of the PV subfamily to the other Enterovirus C subfamilies (see also phylogenetic tree in supplementary file S14). PV2 plays a central role in the exchange of genetic material within the Enterovirus C species.

PVs (see Fig. 6). However, this finding of CV-A13 holds only for a (blast) threshold between 90% and 95% nucleotide identity (see Fig. 6). On the other hand, PV3 shares genetic material with CV-A17, even when applying a very stringent (blast) threshold of > = 95% nucleotide identity. Based on this evolutionary analysis and depending on the threshold applied, it is conceivable that other members of this same evolutionary subgroup, such as CV-A20 and EV-C102 could function as donors of genetic material for PV2, that could in turn circulate it to the other two PVs. *In vitro* and in vivo experiments have shown an ability and a directionality in the exchange of genetic material between PVs and CV-A20 (Jiang et al., 2007). Interestingly, in our analyses, CV-A13 shares genetic material with CV-A24 that belongs to another evolutionary subgroup. The implication of this finding is that other members of the CV-A24 evolutionary subgroup (see Fig. 6 and Supplementary Fig. S14), such as EV-C99, CV-A21, EV-C95, EV-C96 and CV-A11 could possibly share genetic material with CV-A13 and/or CV-A17 that may in turn circulate it to PVs, most probably via PV2. Also, in vitro experiments have demonstrated viable recombinants between PV1 and CV-A21 (Jiang et al., 2007).

The *Enterovirus D* species is divided in 5 genotypes, with the most common of them being EV-D68. Previous analyses have reported intratypic recombination events, but inter-typic recombination events are considered rare (Kyriakopoulou et al., 2014; T. Smura et al., 2007; T.P. Smura et al., 2007; Tan et al., 2015; Yip et al., 2017). This analysis did not observe any recombination events, thus probably reflecting the truly low occurrence of inter-typic recombinations in this species. As more genomes become available, rare events are expected to be detected eventually.

4. Conclusions

Recombination has long been recognized as a driving force for the evolution of enteroviruses. However, due to technological limitations, early studies focused on certain strains, epidemics, partial genomic regions and mostly on phylogenetic incongruences. Advances in genomic technologies have now allowed us to overcome past limitations and study whole genomes from hundreds of different strains. This study exploited all publicly available complete genomes/CDS so as to obtain an unbiased as possible global picture of the patterns of recombination displayed in this particular virus. Despite the plethora of different strains in the four *Enterovirus* species, just a handful of them play a central role as frequent recombination partners. Consequently, their environmental monitoring becomes of paramount importance. Intriguingly, within recombined strains we also observed that 25% (43/170) of recombined sequence fragments do not closely resemble known enteroviral sequences in publicly available databases, even when applying a (blast based) nucleotide identity threshold of 90% (see supplementary file S20). Our interpretation is that there exists a rather large genetic reservoir in the environment, that has the potential to fuel recombination and accelerate the emergence of new *Enterovirus* epidemics. From a genomic perspective, the distal parts of the 5′UTR region and the 2A protein region are highlighted as recombination hotspots in the various genotypes although some other and more restricted studies have identified different hotspots. These two hotspots flank the capsid region, which (as expected from previous studies) exhibits very low recombination rates. Thus, the capsid region emerges as the "psyche" of an enterovirus, whereas other regions seem to be interchangeable. Although we have analyzed a plethora of genomic sequences, it is conceivable that some of the conclusions of this study may

need to be revised in the future, as many more genomes from various geographical locations become available. In addition, there exists a significant bias in sequence databases in favor of certain strains/genotypes that manifest clinical symptoms. In this study, the calculated frequencies of recombination could be considered estimates of the true recombination frequencies that still remain unknown. Also, sequences of different genotypes were determined from samples that have been collected over very different time frames, seasons, populations, or geography, and mass vaccination may have played a role for some but not others. However, several of our conclusions are in accordance with those from previous and independent low/medium-throughput studies, strongly suggesting that this analysis provides a reliable qualitative and quantitative global overview of recombination patterns in the human enteroviruses. Finally, this work provides a computational framework for similar and highly demanding large-scale genome-wide analyses in other frequently recombining viruses.

Acknowledgements

We would like to thank Prof. David L. Robertson, Centre for Virus Research, University of Glasgow, UK, for useful discussions. We would also like to thank Panayotis Vlastaridis for developing the Enterovirus webpage that also contains supplementary material (http://bioinf.bio.uth.gr/enterovirus.html).

Conflict of interest

The authors declare that they have no conflict of interest.

Funding source

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.virol.2018.10.006

References

Akerblom, H.K., Vaarala, O., Hyöty, H., Ilonen, J., Knip, M., 2002. Environmental factors in the etiology of type 1 diabetes. Am. J. Med. Genet. 115, 18–29. https://doi.org/10.1002/ajmg.10340.

Arnold, J.J., Cameron, C.E., 1999. Poliovirus RNA-dependent RNA polymerase (3Dpol) is sufficient for template switching in vitro. J. Biol. Chem. 274, 2706–2716.

Baird, H.A., Galetto, R., Gao, Y., Simon-Loriere, E., Abreha, M., Archer, J., Fan, J., Robertson, D.L., Arts, E.J., Negroni, M., 2006. Sequence determinants of breakpoint location during HIV-1 intersubtype recombination. Nucleic Acids Res. 34, 5203–5216. https://doi.org/10.1093/nar/gkl669.

Benson, D.A., Cavanaugh, M., Clark, K., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., Sayers, E.W., 2013. GenBank. Nucleic Acids Res. 41, D36–D42. https://doi.org/10.1093/nar/gks1195.

Bessaud, M., Joffret, M.-L., Blondel, B., Delpeyroux, F., 2016. Exchanges of genomic domains between poliovirus and other cocirculating species C enteroviruses reveal a high degree of plasticity. Sci. Rep. 6, 38831. https://doi.org/10.1038/srep38831.

Bessaud, M., Joffret, M.-L., Holmblat, B., Razafindratsimandresy, R., Delpeyroux, F., 2011. Genetic relationship between cocirculating Human enteroviruses species C. PLoS One 6, e24823. https://doi.org/10.1371/journal.pone.0024823.

Blomqvist, S., Bruu, A.-L., Stenvik, M., Hovi, T., 2003. Characterization of a recombinant type 3/type 2 poliovirus isolated from a healthy vaccinee and containing a chimeric capsid protein VP1. J. Gen. Virol. 84, 573–580. https://doi.org/10.1099/vir.0.18708-0.

Blomqvist, S., Savolainen-Kopra, C., Paananen, A., El Bassioni, L., El Maamoon Nasr, E.M., Firstova, L., Zamiatina, N., Kutateladze, T., Roivainen, M., 2010. Recurrent isolation of poliovirus 3 strains with chimeric capsid protein Vp1 suggests a recombination hot-spot site in Vp1. Virus Res. 151, 246–251. https://doi.org/10.1016/j.virusres.2010.05.006.

Boros, A., Pankovics, P., Knowles, N.J., Reuter, G., 2012. Natural interspecies recombinant bovine/porcine enterovirus in sheep. J. Gen. Virol. 93, 1941–1951. https://doi.org/10.1099/vir.0.041335-0.

Bouslama, L., Nasri, D., Chollet, L., Belguith, K., Bourlet, T., Aouni, M., Pozzetto, B., Pillet, S., 2007. Natural recombination event within the capsid genomic region leading to a chimeric strain of human enterovirus B. J. Virol. 81, 8944–8952. https://doi.org/10.1128/JVI.00180-07.

Bradrick, S.S., Lieben, E.A., Carden, B.M., Romero, J.R., 2001. A predicted secondary structural domain within the internal ribosome entry site of echovirus 12 mediates a cell-type-specific block to viral replication. J. Virol. 75, 6472–6481. https://doi.org/10.1128/JVI.75.14.6472-6481.2001.

Burns, C.C., Shaw, J., Jorba, J., Bukbuk, D., Adu, F., Gumede, N., Pate, M.A., Abanida, E.A., Gasasira, A., Iber, J., Chen, Q., Vincent, A., Chenoweth, P., Henderson, E., Wannemuehler, K., Naeem, A., Umami, R.N., Nishimura, Y., Shimizu, H., Baba, M., Adeniji, A., Williams, A.J., Kilpatrick, D.R., Oberste, M.S., Wassilak, S.G., Tomori, O., Pallansch, M.A., Kew, O., 2013. Multiple independent emergences of type 2 vaccine-derived polioviruses during a large outbreak in northern Nigeria. J. Virol. 87, 4907–4922. https://doi.org/10.1128/JVI.02954-12.

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., Madden, T.L., 2009. BLAST+: architecture and applications. BMC Bioinforma. 10, 421. https://doi.org/10.1186/1471-2105-10-421.

Chevenet, F., Brun, C., Bañuls, A.-L., Jacq, B., Christen, R., 2006. TreeDyn: towards dynamic graphics and annotations for analyses of trees. BMC Bioinforma. 7, 439. https://doi.org/10.1186/1471-2105-7-439.

Combelas, N., Holmblat, B., Joffret, M.-L., Colbère-Garapin, F., Delpeyroux, F., 2011. Recombination between poliovirus and coxsackie A viruses of species C: a model of viral genetic plasticity and emergence. Viruses 3, 1460–1484. https://doi.org/10.3390/v3081460.

Dedepsidis, E., Kyriakopoulou, Z., Pliaka, V., Markoulatos, P., 2010. Correlation between recombination junctions and RNA secondary structure elements in poliovirus Sabin strains. Virus Genes 41, 181–191. https://doi.org/10.1007/s11262-010-0512-5.

Dedepsidis, E., Pliaka, V., Kyriakopoulou, Z., Brakoulias, C., Levidiotou-Stefanou, S., Pratti, A., Mamuris, Z., Markoulatos, P., 2008. Complete genomic characterization of an intertypic Sabin 3/Sabin 2 capsid recombinant. FEMS Immunol. Med. Microbiol. 52, 343–351. https://doi.org/10.1111/j.1574-695X.2008.00381.x.

Dutkiewicz, M., Stachowiak, A., Swiatkowska, A., Ciesiołka, J., 2016. Structure and function of RNA elements present in enteroviral genomes. Acta Biochim. Pol. 63, 623–630. https://doi.org/10.18388/abp.2016_1337.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461. https://doi.org/10.1093/bioinformatics/btq461.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797. https://doi.org/10.1093/nar/gkh340.

Felsenstein, J., 1974. The evolutionary advantage of recombination. Genetics 78, 737–756.

Gan, Z., Jin, H., Li, J., Yao, X., Zhou, Y., Zhang, X., Zhu, F., 2015. Disease burden of enterovirus 71 in rural central China: a community-based survey. Hum. Vaccin. Immunother. 11, 2400–2405. https://doi.org/10.1080/21645515.2015.1059980.

Gmyl, A.P., Belousov, E.V., Maslova, S.V., Khitrina, E.V., Chetverin, A.B., Agol, V.I., 1999. Nonreplicative RNA recombination in poliovirus. J. Virol. 73, 8958–8965.

Gouy, M., Guindon, S., Gascuel, O., 2010. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol. Biol. Evol. 27, 221–224. https://doi.org/10.1093/molbev/msp259.

Guillot, S., Caro, V., Cuervo, N., Korotkova, E., Combiescu, M., Persu, A., Aubert-Combiescu, A., Delpeyroux, F., Crainic, R., 2000. Natural genetic exchanges between vaccine and wild poliovirus strains in humans. J. Virol. 74, 8434–8443.

Harvala, H., Kalimo, H., Dahllund, L., Santti, J., Hughes, P., Hyypiä, T., Stanway, G., 2002. Mapping of tissue tropism determinants in coxsackievirus genomes. J. Gen. Virol. 83, 1697–1706. https://doi.org/10.1099/0022-1317-83-7-1697.

Jiang, P., Faase, J.A.J., Toyoda, H., Paul, A., Wimmer, E., Gorbalenya, A.E., 2007. Evidence for emergence of diverse polioviruses from C-cluster coxsackie A viruses and implications for global poliovirus eradication. Proc. Natl. Acad. Sci. USA 104, 9457–9462. https://doi.org/10.1073/pnas.0700451104.

Joffret, M.-L., Jégouic, S., Bessaud, M., Balanant, J., Tran, C., Caro, V., Holmblat, B., Razafindratsimandresy, R., Reynes, J.-M., Rakoto-Andrianarivelo, M., Delpeyroux, F., 2012. Common and diverse features of cocirculating type 2 and 3 recombinant vaccine-derived polioviruses isolated from patients with poliomyelitis and healthy children. J. Infect. Dis. 205, 1363–1373. https://doi.org/10.1093/infdis/jis204.

Karakasiliotis, I., Paximadi, E., Markoulatos, P., 2005. Evolution of a rare vaccine-derived multirecombinant poliovirus. J. Gen. Virol. 86, 3137–3142. https://doi.org/10.1099/vir.0.80823-0.

Kew, O., Morris-Glasgow, V., Landaverde, M., Burns, C., Shaw, J., Garib, Z., André, J., Blackman, E., Freeman, C.J., Jorba, J., Sutter, R., Tambini, G., Venczel, L., Pedreira, C., Laender, F., Shimizu, H., Yoneyama, T., Miyamura, T., van Der Avoort, H., Oberste, M.S., Kilpatrick, D., Cochi, S., Pallansch, M., de Quadros, C., 2002. Outbreak of poliomyelitis in Hispaniola associated with circulating type 1 vaccine-derived poliovirus. Science 296, 356–359. https://doi.org/10.1126/science.1068284.

Khetsuriani, N., Quiroz, E.S., Holman, R.C., Anderson, L.J., 2003. Viral meningitis-associated hospitalizations in the United States, 1988–1999. Neuroepidemiology 22, 345–352. https://doi.org/10.1159/000072924.

Kirkegaard, K., Baltimore, D., 1986. The mechanism of RNA recombination in poliovirus. Cell 47, 433–443.

Kitamura, N., Semler, B.L., Rothberg, P.G., Larsen, G.R., Adler, C.J., Dorner, A.J., Emini, E.A., Hanecak, R., Lee, J.J., van der Werf, S., Anderson, C.W., Wimmer, E., 1981. Primary structure, gene organization and polypeptide expression of poliovirus RNA. Nature 291, 547–553.

Korotkova, E., Laassri, M., Zagorodnyaya, T., Petrovskaya, S., Rodionova, E., Cherkasova, E., Gmyl, A., Ivanova, O.E., Eremeeva, T.P., Lipskaya, G.Y., Agol, V.I., Chumakov, K., 2017. Pressure for pattern-specific intertypic recombination between sabin poliovirus: evolutionary implications. Viruses 9. https://doi.org/10.3390/v9110353.

Krogstad, P., Hammon, R., Halnon, N., Whitton, J.L., 2008. Fatal neonatal myocarditis caused by a recombinant human enterovirus-B variant. Pediatr. Infect. Dis. J. 27, 668–669. https://doi.org/10.1097/INF.0b013e31816b5d2a.

Kyriakopoulou, Z., Amoutzias, G.D., Dimitriou, T.G., Tsakogiannis, D., Mossialos, D., Markoulatos, P., 2017. Intra- and inter-serotypic recombinations in the 5′ UTR-VP4 region of Echovirus 30 strains. Arch. Virol. https://doi.org/10.1007/s00705-017-3600-1.

Kyriakopoulou, Z., Kottaridi, C., Dedepsidis, E., Bolanaki, E., Levidiotou-Stefanou, S., Markoulatos, P., 2006. Molecular characterization of wild-type polioviruses isolated in Greece during the 1996 outbreak in Albania. J. Clin. Microbiol. 44, 1150–1152. https://doi.org/10.1128/JCM.44.3.1150-1152.2006.

Kyriakopoulou, Z., Pliaka, V., Amoutzias, G.D., Markoulatos, P., 2014. Recombination among human non-polio enteroviruses: implications for epidemiology and evolution. Virus Genes. https://doi.org/10.1007/s11262-014-1152-y.

Lin, C.-H., Wang, Y.-B., Chen, S.-H., Hsiung, C.A., Lin, C.-Y., 2015. Precise genotyping and recombination detection of Enterovirus. BMC Genom. 16 (Suppl. 12), S8. https://doi.org/10.1186/1471-2164-16-S12-S8.

Lindberg, A.M., Andersson, P., Savolainen, C., Mulders, M.N., Hovi, T., 2003. Evolution of the genome of Human enterovirus B: incongruence between phylogenies of the VP1 and 3CD regions indicates frequent recombination within the species. J. Gen. Virol. 84, 1223–1235. https://doi.org/10.1099/vir.0.18971-0.

Liu, D.-P., Wang, T.-A., Huang, W.-T., Chang, L.-Y., Wang, E.-T., Cheng, S.-H., Yang, M.-C., 2016. Disease burden of enterovirus infection in Taiwan: implications for vaccination policy. Vaccine 34, 974–980. https://doi.org/10.1016/j.vaccine.2015.12.026.

Lowry, K., Woodman, A., Cook, J., Evans, D.J., 2014. Recombination in enteroviruses is a biphasic replicative process involving the generation of greater-than genome length "imprecise" intermediates. PLoS Pathog. 10, e1004191. https://doi.org/10.1371/journal.ppat.1004191.

Lukashev, A.N., 2005. Role of recombination in evolution of enteroviruses. Rev. Med. Virol. 15, 157–167. https://doi.org/10.1002/rmv.457.

Lukashev, A.N., Lashkevich, V.A., Ivanova, O.E., Koroleva, G.A., Hinkkanen, A.E., Ilonen, J., 2005. Recombination in circulating Human enterovirus B: independent evolution of structural and non-structural genome regions. J. Gen. Virol. 86, 3281–3290. https://doi.org/10.1099/vir.0.81264-0.

Lukashev, A.N., Shumilina, E.Y., Belalov, I.S., Ivanova, O.E., Eremeeva, T.P., Reznik, V.I., Trotsenko, O.E., Drexler, J.F., Drosten, C., 2014. Recombination strategies and evolutionary dynamics of the Human enterovirus A global gene pool. J. Gen. Virol. 95, 868–873. https://doi.org/10.1099/vir.0.060004-0.

Magiorkinis, G., Paraskevis, D., Vandamme, A.-M., Magiorkinis, E., Sypsa, V., Hatzakis, A., 2003. In vivo characteristics of human immunodeficiency virus type 1 intersubtype recombination: determination of hot spots and correlation with sequence similarity. J. Gen. Virol. 84, 2715–2722. https://doi.org/10.1099/vir.0.19180-0.

Malim, M.H., Emerman, M., 2001. HIV-1 sequence variation: drift, shift, and attenuation. Cell 104, 469–472.

McIntyre, C.L., Savolainen-Kopra, C., Hovi, T., Simmonds, P., 2013. Recombination in the evolution of human rhinovirus genomes. Arch. Virol. 158, 1497–1515. https://doi.org/10.1007/s00705-013-1634-6.

McWilliam Leitch, E.C., Cabrerizo, M., Cardosa, J., Harvala, H., Ivanova, O.E., Koike, S., Kroes, A.C.M., Lukashev, A., Perera, D., Roivainen, M., Susi, P., Trallero, G., Evans, D.J., Simmonds, P., 2012. The association of recombination events in the founding and emergence of subgenogroup evolutionary lineages of human enterovirus 71. J. Virol. 86, 2676–2685. https://doi.org/10.1128/JVI.06065-11.

Mirand, A., Henquell, C., Archimbaud, C., Peigue-Lafeuille, H., Bailly, J.-L., 2007. Emergence of recent echovirus 30 lineages is marked by serial genetic recombination events. J. Gen. Virol. 88, 166–176. https://doi.org/10.1099/vir.0.82146-0.

Muehlenbachs, A., Bhatnagar, J., Zaki, S.R., 2015. Tissue tropism, pathology and pathogenesis of enterovirus infection. J. Pathol. 235, 217–228. https://doi.org/10.1002/path.4438.

Muller, H.J., 1964. The relation of recombination to mutational advance. Mutat. Res. 106, 2–9.

Muslin, C., Joffret, M.-L., Pelletier, I., Blondel, B., Delpeyroux, F., 2015. Evolution and emergence of enteroviruses through intra- and inter-species recombination: plasticity and phenotypic impact of modular genetic exchanges in the 5′ untranslated region. PLoS Pathog. 11, e1005266. https://doi.org/10.1371/journal.ppat.1005266.

Nora, T., Charpentier, C., Tenaillon, O., Hoede, C., Clavel, F., Hance, A.J., 2007. Contribution of recombination to the evolution of human immunodeficiency viruses expressing resistance to antiretroviral treatment. J. Virol. 81, 7620–7628. https://doi.org/10.1128/JVI.00083-07.

Oberste, M.S., Maher, K., Kilpatrick, D.R., Pallansch, M.A., 1999. Molecular evolution of the human enteroviruses: correlation of serotype with VP1 sequence and application to picornavirus classification. J. Virol. 73, 1941–1948.

Oberste, M.S., Peñaranda, S., Pallansch, M.A., 2004. RNA recombination plays a major role in genomic change during circulation of coxsackie B viruses. J. Virol. 78, 2948–2955.

Oprisan, G., Combiescu, M., Guillot, S., Caro, V., Combiescu, A., Delpeyroux, F., Crainic, R., 2002. Natural genetic recombination between co-circulating heterotypic enteroviruses. J. Gen. Virol. 83, 2193–2200. https://doi.org/10.1099/0022-1317-83-9-2193.

Pallansch, M., Roos, R., 2007. Enteroviruses: Polioviruses, Coxsackieviruses, Echoviruses, and Newer Enteroviruses, 5th ed. Lippincott Williams & Wilkins, Philadelphia (Fields virology).

Parasuraman, T.V., Frenia, K., Romero, J., 2001. Enteroviral meningitis. Cost of illness and considerations for the economic evaluation of potential therapies. Pharmacoeconomics 19, 3–12.

Paximadi, E., Karakasiliotis, I., Bolanaki, E., Krikelis, A., Markoulatos, P., 2007. Vaccine derived bi- and multi-recombinant Sabin strains. Virus Genes 35, 541–548. https://doi.org/10.1007/s11262-007-0146-4.

Pelletier, J., Sonenberg, N., 1988. Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. Nature 334, 320–325. https://doi.org/10.1038/334320a0.

Pliaka, V., Kyriakopoulou, Z., Markoulatos, P., 2012. Risks associated with the use of live-attenuated vaccine poliovirus strains and the strategies for control and eradication of paralytic poliomyelitis. Expert Rev. Vaccin. 11, 609–628. https://doi.org/10.1586/erv.12.28.

Puenpa, J., Vongpunsawad, S., Österback, R., Waris, M., Eriksson, E., Albert, J., Midgley, S., Fischer, T.K., Eis-Hübinger, A.M., Cabrerizo, M., Gaunt, E., Simmonds, P., Poovorawan, Y., 2016. Molecular epidemiology and the evolution of human coxsackievirus A6. J. Gen. Virol. 97, 3225–3231. https://doi.org/10.1099/jgv.0.000619.

Rakoto-Andrianarivelo, M., Guillot, S., Iber, J., Balanant, J., Blondel, B., Riquet, F., Martin, J., Kew, O., Randriamanalina, B., Razafinimpiasa, L., Rousset, D., Delpeyroux, F., 2007. Co-circulation and evolution of polioviruses and species C enteroviruses in a district of Madagascar. PLoS Pathog. 3, e191. https://doi.org/10.1371/journal.ppat.0030191.

Runckel, C., Westesson, O., Andino, R., DeRisi, J.L., 2013. Identification and manipulation of the molecular determinants influencing poliovirus recombination. PLoS Pathog. 9, e1003164. https://doi.org/10.1371/journal.ppat.1003164.

Santti, J., Hyypiä, T., Kinnunen, L., Salminen, M., 1999. Evidence of recombination among enteroviruses. J. Virol. 73, 8741–8749.

Savolainen-Kopra, C., Blomqvist, S., 2010. Mechanisms of genetic variation in polioviruses. Rev. Med. Virol. 20, 358–371. https://doi.org/10.1002/rmv.663.

Simmonds, P., Welch, J., 2006. Frequency and dynamics of recombination within different species of human enteroviruses. J. Virol. 80, 483–493. https://doi.org/10.1128/JVI.80.1.483-493.2006.

Simon-Loriere, E., Holmes, E.C., 2011. Why do RNA viruses recombine? Nat. Rev. Microbiol. 9, 617–626. https://doi.org/10.1038/nrmicro2614.

Simon-Loriere, E., Martin, D.P., Weeks, K.M., Negroni, M., 2010. RNA structures facilitate recombination-mediated gene swapping in HIV-1. J. Virol. 84, 12675–12682. https://doi.org/10.1128/JVI.01302-10.

Smura, T., Blomqvist, S., Paananen, A., Vuorinen, T., Sobotová, Z., Bubovica, V., Ivanova, O., Hovi, T., Roivainen, M., 2007. Enterovirus surveillance reveals proposed new serotypes and provides new insight into enterovirus 5′-untranslated region evolution. J. Gen. Virol. 88, 2520–2526. https://doi.org/10.1099/vir.0.82866-0.

Smura, T.P., Junttila, N., Blomqvist, S., Norder, H., Kaijalainen, S., Paananen, A., Magnius, L.O., Hovi, T., Roivainen, M., 2007. Enterovirus 94, a proposed new serotype in human enterovirus species D. J. Gen. Virol. 88, 849–858. https://doi.org/10.1099/vir.0.82510-0.

Stanway, G., 2013. Molecular biology and classification of enteroviruses. In: Taylor, K., Hyöty, H., Toniolo, A., Zuckerman, A.J. (Eds.), Diabetes and Viruses. Springer, New York, New York, NY, pp. 109–115. https://doi.org/10.1007/978-1-4614-4051-2_12.

Stern, A., Yeh, M.T., Zinger, T., Smith, M., Wright, C., Ling, G., Nielsen, R., Macadam, A., Andino, R., 2017. The evolutionary pathway to virulence of an RNA virus. Cell 169 (35–46), e19. https://doi.org/10.1016/j.cell.2017.03.013.

Su, G., Morris, J.H., Demchak, B., Bader, G.D., 2014. Biological network exploration with Cytoscape 3 (8.13.1-24). Curr. Protoc. Bioinforma. 47. https://doi.org/10.1002/0471250953.bi0813s47.

Tan, Y., Hassan, F., Schuster, J.E., Simenauer, A., Selvarangan, R., Halpin, R.A., Lin, X., Fedorova, N., Stockwell, T.B., Lam, T.T.-Y., Chappell, J.D., Hartert, T.V., Holmes, E.C., Das, S.R., 2015. Molecular evolution and intraclade recombination of enterovirus D68 during the 2014 outbreak in the United States. J. Virol. 90, 1997–2007. https://doi.org/10.1128/JVI.02418-15.

Tsimpidis, M., Bachoumis, G., Mimouli, K., Kyriakopoulou, Z., Robertson, D.L., Markoulatos, P., Amoutzias, G.D., 2017. T-RECs: rapid and large-scale detection of recombination events among different evolutionary lineages of viral genomes. BMC Bioinforma. 18, 13. https://doi.org/10.1186/s12859-016-1420-z.

Whitton, J.L., Cornell, C.T., Feuer, R., 2005. Host and virus determinants of picornavirus pathogenesis and tropism. Nat. Rev. Microbiol. 3, 765–776. https://doi.org/10.1038/nrmicro1284.

Wimmer, E., Hellen, C.U., Cao, X., 1993. Genetics of poliovirus. Annu. Rev. Genet. 27, 353–436. https://doi.org/10.1146/annurev.ge.27.120193.002033.

Xiao, H., Huang, K., Li, L., Wu, X., Zheng, L., Wan, C., Zhao, W., Ke, C., Zhang, B., 2014. Complete genome sequence analysis of human echovirus 30 isolated during a large outbreak in Guangdong Province of China, in 2012. Arch. Virol. 159, 379–383. https://doi.org/10.1007/s00705-013-1818-0.

Xiao, Y., Rouzine, I.M., Bianco, S., Acevedo, A., Goldstein, E.F., Farkov, M., Brodsky, L., Andino, R., 2016. RNA recombination enhances adaptability and is required for virus spread and virulence. Cell Host Microbe 19, 493–503. https://doi.org/10.1016/j.chom.2016.03.009.

Yip, C.C.Y., Lo, J.Y.C., Sridhar, S., Lung, D.C., Luk, S., Chan, K.-H., Chan, J.F.W., Cheng, V.C.C., Woo, P.C.Y., Yuen, K.-Y., Lau, S.K.P., 2017. First report of a fatal case associated with EV-D68 Infection in Hong Kong and emergence of an interclade recombinant in China revealed by genome analysis. Int. J. Mol. Sci. 18. https://doi.org/10.3390/ijms18051065.

Yogarajah, T., Ong, K.C., Perera, D., Wong, K.T., 2017. AIM2 inflammasome-mediated pyroptosis in enterovirus A71-infected neuronal cells restricts viral replication. Sci. Rep. 7, 5845. https://doi.org/10.1038/s41598-017-05589-2.

Yozwiak, N.L., Skewes-Cox, P., Gordon, A., Saborio, S., Kuan, G., Balmaseda, A., Ganem, D., Harris, E., DeRisi, J.L., 2010. Human enterovirus 109: a novel interspecies recombinant enterovirus isolated from a case of acute pediatric respiratory illness in Nicaragua. J. Virol. 84, 9047–9058. https://doi.org/10.1128/JVI.00698-10.

Zell, R., Delwart, E., Gorbalenya, A.E., Hovi, T., King, A.M.Q., Knowles, N.J., Lindberg, A.M., Pallansch, M.A., Palmenberg, A.C., Reuter, G., Simmonds, P., Skern, T., Stanway, G., Yamashita, T., 2017. ICTV virus taxonomy profile: picornaviridae (Ictv Report Consortium, null). J. Gen. Virol. https://doi.org/10.1099/jgv.0.000911.

Zhang, J., Temin, H.M., 1994. Retrovirus recombination depends on the length of sequence identity and is not error prone. J. Virol. 68, 2409–2414.

Zhang, J., Temin, H.M., 1993. Rate and mechanism of nonhomologous recombination during a single cycle of retroviral replication. Science 259, 234–238.

Zhang, Y., Zhu, S., Yan, D., Liu, G., Bai, R., Wang, D., Chen, L., Zhu, H., An, H., Kew, O., Xu, W., 2010. Natural type 3/type 2 intertypic vaccine-related poliovirus recombinants with the first crossover sites within the VP1 capsid coding region. PLoS One 5, e15300. https://doi.org/10.1371/journal.pone.0015300.