Virus Research 147 (2010) 91–97

Contents lists available at ScienceDirect

Virus Research

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

Outbreak of poliomyelitis in Finland in 1984–85 – Re-analysis of viral sequences using the current standard approach

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ARTICLE INFO

Article history:
Received 23 June 2009
Received in revised form 16 October 2009
Accepted 22 October 2009
Available online 31 October 2009

Keywords:
Poliovirus
Sequence variation
Epidemics

ABSTRACT

In 1984, a wild type 3 poliovirus (PV3/FIN84) spread all over Finland causing nine cases of paralytic poliomyelitis and one case of aseptic meningitis. The outbreak was ended in 1985 with an intensive vaccination campaign. By limited sequence comparison with previously isolated PV3 strains, closest relatives of PV3/FIN84 were found among strains circulating in the Mediterranean region. Now we wanted to reanalyse the relationships using approaches currently exploited in poliovirus surveillance. Cell lysates of 22 strains isolated during the outbreak and stored frozen were subjected to RT-PCR amplification in three genomic regions without prior subculture. Sequences of the entire VP1 coding region, 150 nucleotides in the VP1-2A junction, most of the 5′ non-coding region, partial sequences of the 3D RNA polymerase coding region and partial 3′ non-coding region were compared within the outbreak and with sequences available in data banks. In addition, complete nucleotide sequences were obtained for 2 strains isolated from two different cases of disease during the outbreak. The results confirmed the previously described wide intraepidemic variation of the strains, including amino acid substitutions in antigenic sites, as well as the likely Mediterranean region origin of the strains. Simplot and bootscanning analyses of the complete genomes indicated complicated evolutionary history of the non-capsid coding regions of the genome suggesting several recombinations with different HEV-C viruses in the past.

1. Introduction

Finland had been free of poliomyelitis for 20 years until a surprising outbreak occurred between August 1984 and January 1985. The epidemic poliovirus type 3 (PV3/FIN84) was found to circulate all over the country and caused nine cases of paralytic poliomyelitis and one case of aseptic meningitis. The outbreak was ended with an intensive vaccination campaign including one dose of inactivated poliovirus vaccine (IPV) to children aged 18 years or younger followed by a dose of trivalent oral poliovirus vaccine to the entire population of Finland (Hovi et al., 1986).

The complete genome of one epidemic strain, isolated from a healthy contact of the first paralytic patient, was sequenced (Hughes et al., 1986). Genetic and antigenic variations between different PV3/FIN84 strains were subsequently reported in extensive studies (Kinnunen et al., 1991; Huovilainen et al., 1988). By limited sequence comparison with previously isolated PV3 strains, closest relatives were found among strains circulating in the Mediterranean region (Poyry et al., 1990). Genetic relationships between strains were determined using relatively short sequences.

We wanted to reanalyse some of the relationships using approaches currently exploited in the World Health Organization coordinated poliovirus surveillance. We also considered it possible that two decades' intensive poliovirus surveillance throughout the world might have revealed closer relatives to the PV3/FIN84 strains than those described before. The genetic relationships to other strains based on the complete VP1 region are described. We also present complete nucleotide sequences of 2 strains isolated from two different cases of disease.

2. Materials and methods

2.1. Virus strains

Twenty two virus strains studied (Table 1) had been isolated from faecal samples during the outbreak in 1984–85 (Kinnunen et al., 1986; Hovi et al., 1986). The isolates were kept in −20 °C freezer during the years and used in this study without further passaging. Seventeen of the strains were derived from eight different patients: six of them had paralytic poliomyelitis, one had aseptic meningitis

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doi:10.1016/j.virusres.2009.10.012

and one had a transient attack of convulsions. Five strains were derived from three healthy excretors, and two of these strains were known not to be related to the epidemic PV3.

2.2. RNA isolation and reverse transcriptase polymerase chain reaction (RT-PCR)

Viral RNA was isolated from 100 μl of virus-positive cell culture suspension using E.Z.N.A.® Total RNA kit (Omega Bio-Tek, Doraville, GA, USA). RT-PCRs for the complete VP1 region of 22 strains, partial 5′NCR, 3′NCR and 3D regions of 20 epidemic strains, as well as the complete genome of two epidemic strains (see below), were performed using the primer pairs shown in Supplementary Table 1 (online). Primers used were designed for the current study with the exceptions (marked in table) of primers described in El Bassioni et al. (2003), Pulli et al. (1995) and Savolainen et al. (2004). Primers for 5′NCR were previously designed in our laboratory for alignment containing all EV prototypes. Excluding primers, RT-PCRs for genomic regions other than 5′NCR were carried out under conditions described by Oberste et al. (2003). The reactions for 5′NCR were incubated at 50 °C for 30 min, followed by 94 °C for 4 min. Thermocycling was performed for 40 cycles, each including 15 s at 94 °C, 30 s at 50 °C, and 90 s at 60 °C. Thermocycling was followed by incubation for 5 min at 72 °C. The reaction mixture for 5′NCR RT-PCR included 2 μl of 5 μM primer per reaction. PCR products were visualized after electrophoresis on ethidium bromide-stained 2% agarose gels.

2.3. Sequence and phylogenetic analyses

PCR products were purified prior to sequencing either directly with PCR Purification kit (QIAquick, Qiagen GmbH, Hilden, Germany), or in case of multiple bands, excised from 1% agarose gel and purified with the QIAquick Gel Extraction kit according to the manufacturer's instructions (Qiagen). Alternatively, Elchrom Gel Purification kit (Elchrom Scientific AG, Cham, Switzerland) was used. The purified products were stored at −20 °C until they were sent for sequencing to Molecular Medicine Sequencing Laboratory (National Public Health Institute, Helsinki, Finland) with the same primer pairs as used in RT-PCR. Nucleotide sequences of the following regions were defined (numbering according to PV3/FIN84/23127 in Hughes et al., 1986): partial 5′NCR (nucleotides 15–536; 522 nt), complete VP1 (nucleotides 2481–3380; 900 nt), VP1-2A junction (nucleotides 3291–3440; 150 nt), partial 3D-3′NCR (nucleotides 6734–7405; 672 nt except for strain PA23364 nucleotides, 6734–7194; 461 nt due to a weak PCR product of the 3′ end of the region). Complete genome sequences of two selected strains (see below) were also determined. Short stretches of nucleotides in both ends of the genome remained unidentified. The term "complete genome sequence" is used for simplicity throughout the manuscript. The sequences generated in this study have been submitted to GenBank under accession numbers FJ842158–FJ842260.

Nucleotide sequence data were analysed with ContigExpress (Vector NTI Advance 10.1; Invitrogen Corporation, Carlsbad, CA, USA, 2005) and multiple sequence alignments were made using AlignX (Vector NTI Advance 10.1) or MEGA version 4.0. (Tamura et al., 2007). Sequenced regions were analysed by comparing 20 epidemic strains of each region with each other and with the corresponding region of strain PV3/FIN84/23127. A consensus sequence showing the most common base for each nucleotide position was constructed for every genomic region. Nucleotide sequences were compared to sequences in GenBank using BLAST nucleotide–nucleotide program (http://blast.ncbi.nlm.nih.gov/blast/Blast.cgi). Phylogenetic trees were produced and visualized using the neighbor-joining method with MEGA version 4.0. The bootstrap analysis was performed using 1000 replicates. The transition–transversion bias was estimated from the data (3.3 for the VP1 dataset including all sequences in the phylogenetic analysis), and the evolutionary dis-

Table 1
PV3 epidemic in Finland in 1984–1985 – background information of strains.

Virus strain	Patient no.[a]	Code	Gender	Age (y)	Clinical symptoms	Epidemiological connections	Location
60212 25725-2920 25870-3999 814-8427 814	1	AK	M	6	Aseptic meningitis	Brother of SK	Vantaa (Helsinki region)
801-8238	2	OI	M	17	Paralytic poliomyelitis	Contact with SJ	Vuolijoki (Northern Finland)
34447-26108 26107-34384 26101-12785 26108-34401	3	KY	M	12	Paralytic poliomyelitis		Oulu (Northern Finland)
26063-12528	4	RA	M	4	Paralytic poliomyelitis		Naantali (Southern Finland)
2493	5	KE	F	31	Paralytic poliomyelitis		Helsinki
2575 32997	6	RU	M	26	Paralytic poliomyelitis		Tampere (Middle Finland)
1460	7	SA	M	33	Paralytic poliomyelitis		Helsinki
25959	NR[b]	SK	F	?	Healthy	Sister of patient no. 1	Vantaa (Helsinki region)
25916-6674 26075-33677	NR[b]	SJ	F	?	Healthy	Contact with patient no. 2	Kajaani (Northern Finland)
23364 23390	NC[c]	PA	F	?	Transient attack of convulsions		Haukipudas (Northern Finland)
77-32899 349-1848	NR[b]	RO	F	?	Healthy	Immigrant from Ethiopia	Helsinki

[a] According to Kinnunen et al. (1986).
[b] NR, not relevant.
[c] NC, not classified as polio patient during the epidemic.