Infection (2013) 41:231–235
DOI 10.1007/s15010-012-0340-8

CASE REPORT

Family outbreak of an infection with a recombinant Coxsackie A virus in eastern Switzerland

R. Butsch · C. Tapparel · P. Keller · K. Herzog · M. Krause · W. Wunderli · L. Kaiser · W. Bossart

Received: 15 March 2012 / Accepted: 12 September 2012 / Published online: 5 October 2012

Abstract
Purpose We report on an unusual familial outbreak of a coxsackie virus infection in Switzerland in which five family members were affected. Most of the patients presented with signs of meningitis, and four were hospitalized.
Methods In three individuals, the virus was detected in the cerebrospinal fluid, pharynx, and stool, respectively. The genome was sequenced in specimens of two patients.
Results The nucleotide sequences of both virus strains were identical. Blast search revealed that the first half of the sequence was 88 % homologous to Enterovirus 75 (EV-75), 87 % with Echovirus 11 (E-11), and 84 % homologous to Coxsackie virus A9 (CV-A9). The second half of the sequence was 77 % homologous to EV-75, 75 % to E-11, and 91 % to CV-A9.
Conclusion We propose that the isolated virus strain is a recombinant strain with a 5′ untranslated region and with the start of the VP4 sequence originating from E-11/EV-75 and the rest of the genome originating from CV-A9. Interestingly, this novel virus strain showed an exceptional virulence and rapid spread. Two weeks after the initial outbreak in this family, a similar outbreak was observed in a second geographic area roughly 100 km distant to the primary identification site, and another 2 months later this virus strain was found to circulate in the western part of Switzerland some 250 km distant to the primary locus. These findings suggest that genetic recombination has resulted in a novel enterovirus with features of high virulence, contagiosity, and spreading.

R. Butsch (✉)
Division of Internal Medicine, University Hospital Zurich, Raemistrasse 100, 8091 Zurich, Switzerland
e-mail: raphael.butsch@usz.ch

R. Butsch · M. Krause
Department of Internal Medicine, Cantonal Hospital Münsterlingen, 8596 Münsterlingen, Switzerland

M. Krause
e-mail: martin.krause@stgag.ch

C. Tapparel · L. Kaiser
Laboratory of Virology, Division of Infectious Diseases and Division of Laboratory Medicine, University of Geneva Hospitals, 4 Rue Gabrielle-Perret-Gentil, 1211 Geneva 14, Switzerland
e-mail: caroline.tapparel@hcuge.ch

L. Kaiser
e-mail: laurent.kaiser@hcuge.ch

P. Keller
Institute of Medical Microbiology, University Laboratories, Friedrich Schiller University of Jena, Erlanger Allee 101, 07747 Jena, Germany
e-mail: peter.keller@med.uni-jena.de

K. Herzog
Department of Microbiology, Cantonal Hospital Münsterlingen, P.O. Box, 8596 Münsterlingen, Switzerland
e-mail: kathrin.herzog@stgag.ch

W. Wunderli · W. Bossart
Institute of Medical Virology, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland
e-mail: wernerwunderli@bluewin.ch

W. Bossart
e-mail: bossart.walter@virology.uzh.ch

Keywords Coxsackie virus · Enterovirus · Outbreak · Aseptic meningitis · Recombination

Introduction

Human enteroviruses are taxonomically split into four different species (A–D), with the different coxsackie viruses being classified into Enterovirus A, B, and C species and the echoviruses into Enterovirus B species [1, 2].

Coxsackie viruses induce a wide variety of clinical symptoms, including aseptic meningitis, myocarditis, herpangina, myalgia, flu-like respiratory disease ("summer flu"), pleurodynia, hepatitis, diarrhea, and exanthema including hand-foot-and-mouth disease. Clinical symptoms largely depend on the virus type as well as on patient age and immune status. In contrast, echoviruses are typically associated with febrile illnesses which may include rashes and respiratory diseases. Altogether, most infections caused by non-polio enteroviruses result only in mild febrile illness [3].

Recombination between different enteroviruses was described for polioviruses as early as 1962 [4, 5], and it was later also confirmed for non-polioviruses and proven to occur frequently [2, 6–12]. The researchers conducting these studies speculated that recombination contributes to the evolution of new genetic virus lineages with novel properties.

We report here an unusual family outbreak of a recombinant Coxsackie A9 virus with high incidence of acute meningitis and rapid spread within Switzerland.

Materials and methods

Patients

We report on a family of five members living next to Lake Constance in eastern Switzerland. The father, mother, and two children were living together in one household. An uncle from outside frequently took care of the children. All five family members fell sick and were suffering from a febrile infection with strong headache. The first to fall ill was the younger of the two children, a 16-month-old boy, followed by his uncle, father, brother and mother. All individuals except for the mother were hospitalized (Table 1).

Diagnostic methods

Virus isolation and immunofluorescence

Virus isolation attempts succeeded in two specimens of stool on Rita cells, but failed on Vero, A549, MRC5, and Caco 2 cells as well as in the throat swap specimen. The isolated cytopathogenic virus was subtyped as a Coxsackie A9 virus by using a set of anti-enterovirus monoclonal antibodies (Chemicon, Temecula, CA).

RNA extraction, reverse transcription-PCR and real-time PCR

RNA was extracted from clinical specimens, such as cerebrospinal fluid (CSF), swabs, and stool by using the

Table 1 Clinical features and microbiological findings of the five affected family members

Case	Relationship	Age	Main symptoms (onset)	Microbiological investigations		
				Material	Method	Result
1	Younger boy	16 months	Fever, cough, coryza (day 0)	Smear: pharynx	Cell culture	Enterovirus negative
					Immunofluorescence	Enterovirus antigen negative
				Stool	Cell culture	Enterovirus negative
					Immunofluorescence	Enterovirus antigen negative
2	Uncle (paternal side)	31 years	Headache, fever, vomiting (day 0)	CSF	RT-PCR	Enterovirus RNA positive
				Smear: pharynx	RT-PCR	Enterovirus RNA positive
					Cell culture	Enterovirus negative
3	Father	43 years	Headache, fatigue, vomiting (day 4)	CSF	RT-PCR	Enterovirus RNA positive
				Stool	RT-PCR	Enterovirus RNA positive
					Cell culture	Enterovirus positive
4	Older boy	3 years	Fever, headache, coryza, rash, abdominal pain (day 6)	CSF	RT-PCR	Enterovirus RNA positive
				Smear: pharynx	Immunofluorescence	Enterovirus antigen negative
					Cell culture	Enterovirus negative
				Stool	Immunofluorescence	Enterovirus Antigen negative
					Cell culture	Enterovirus positive
5	Mother	36 years	Headache, fever, nausea (day 10)	Not done	Not available	Not available

CSF cerebrospinal fluid, *RT* reverse transcriptase

NucliSens easyMAG™ nucleic acid extraction system and reagents (Biomérieux, Marcy-l'Etoile, France). Reverse transcribed (RT) RNA (Superscript II; Invitrogen, Carlsbad CA) was then assayed with the Entero/Ge/08 real-time PCR assay using oligonucleotide sequences and amplification protocol previously described [13]. For genotyping, cDNA was amplified with forward (5′-CCC CTG AAT GCG GCT AAT-3′) and reverse primers (ATCHGGHA RYTTCCAMCACCA). All PCR products were purified by passage through Microcon spin columns (Millipore, Billerica MA) and sequenced in the ABI Prism 3130XL DNA Sequencer (Applied Biosystems, Foster City, CA). Chromatograms were imported for proofreading with the Geneious Pro software (Biomatters, Queensland, New Zealand), and the sequences are available at Genbank (accession numbers JQ037833–JQ037835).

Phylogenetic analysis

Trees were built with the PhyML software program using the generalized time-reversible model and optimized tree topology and branch lengths [14] (see Fig. 1). The transition/transversion ratio and relative substitution rate categories were set to 4 and 8, respectively. The gamma shape parameter alpha and proportions of invariant sites were estimated from the data.

Results

Case descriptions

The clinical features of the five affected family members and the timely relationship are summarized in Table 1.

Case 1

The first family member to fall ill was the 16-month-old son who developed fever up to 39.3 °C with coryza and cough. His stool was loose but diarrhea was not present. He was hospitalized as a precaution together with his relatives 10 days after the onset of clinical symptoms. At initial exam, he had partly recovered, was in good general condition, afebrile, with a heart rate of 125 beats per minute (bpm) and a respiratory rate of 26 breaths per minute. Apart from rhinitis, no other clinical signs of infection were found. No signs of meningitis were present. Laboratory analysis showed a leukocytosis of 22,100 cells/µl (66 % mononuclear) and C-reactive protein (CRP) of 4 mg/l. Cough and rhinitis vanished within another 2 days, and he was discharged from the hospital. The search for enterovirus by cell culture and RT-PCR in stool and pharyngeal secretions was negative.

Case 2

The 31-year-old uncle fell sick at about the same time as case 1 with rhinitis, sore throat, cough and fever up to 38.8 °C. One week later he developed headache and vomiting. On admission 1 day later, his temperature was 36.5 °C, blood pressure was 140/80 mmHg, and his heart rate was 76 bpm. General and neurological examinations were normal without signs of meningitis. Leukocytes were at 11,900/µl and CRP at 12 mg/l. Analysis of the CSF showed that he had 12 cells/µl (99 % mononuclear cells). Cultures from CSF and blood as well as a PCR assay for herpes simplex virus Type 1 and 2 were negative. RT-PCR analysis of the CSF and pharyngeal secretions revealed the presence of enterovirus. The patient recovered slowly and was able to leave the hospital after 3 days.

Case 3

Of all family members, the 43-year-old father suffered the most from infection, resulting in a high level of disability. The symptoms had evolved over a period of several days, beginning 4 days later than those of case 1. Another 4 days later he was hospitalized, 2 days before his relatives, because of severe persistent headache with nausea and vomiting. Photophobia and phonophobia had also developed. Neurologic examination, laboratory findings, and magnetic resonance imaging (MRI) of the brain were normal. His temperature was 36.6 °C, blood pressure was 136/80 mmHg, and his heart rate was 80 bpm. Clinical examinations as well as routine laboratory analyses were normal. Analysis of the CSF revealed 218 cells/µl, with 100 % mononuclear cells. The presence of enterovirus was demonstrated by RT-PCR in the CSF and stool, as well as by cell culture in stool. Other cultures of CSF and blood remained negative, as well as a PCR assay for herpes simplex virus type 1 and 2 and serologic examinations for Epstein–Barr virus, cytomegalovirus, *Borrelia burgdorferi*, and tick-borne encephalitis (FSME). This patient was discharged from hospital after 5 days, but continued to suffer from residual pain and fatigue for a further 2 months.

Case 4

The second son, a 3-year-old toddler, became ill 6 days after case 1. He suffered from headache, cough, abdominal pain, an itching rash, and fever of 39 °C. He was hospitalized together with the brother and the uncle another 3 days later. Rhinitis, a slight redness of the pharynx and the left drumhead, as well as pronounced borborygmi were noted. Blood analysis revealed 12,900 leukocytes/µl, and the CRP was <1 mg/l. The cell count in the CSF was distinctly elevated with 490 cells/µl (95 % were

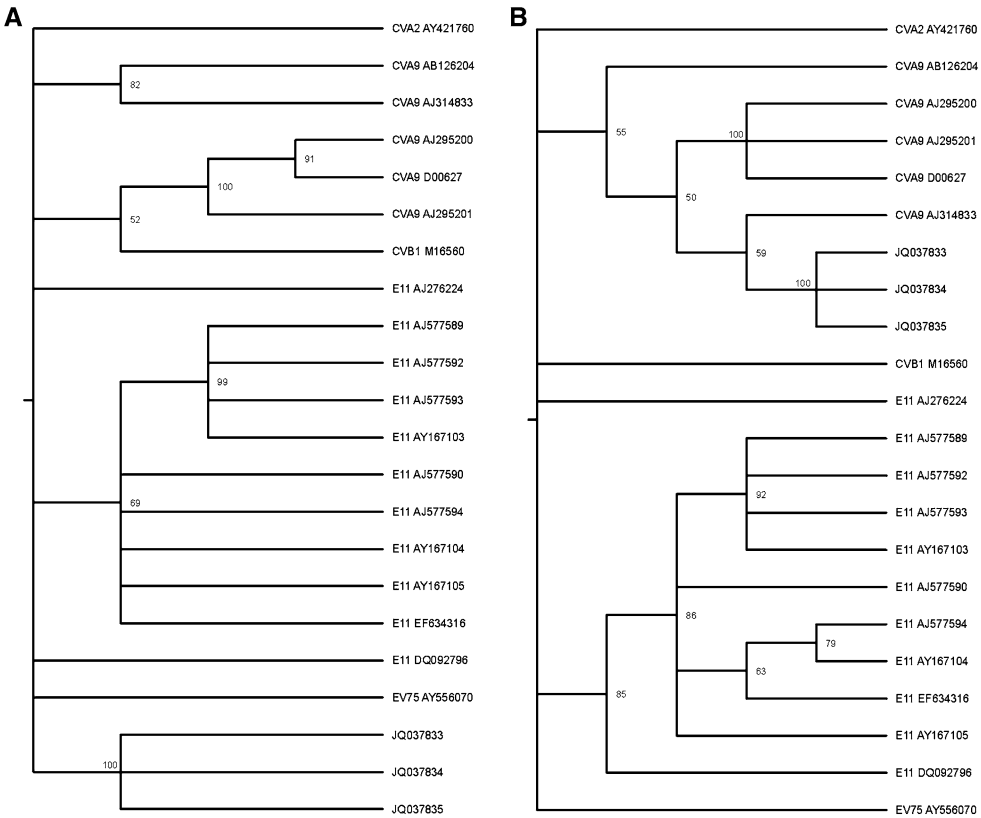

Fig. 1 Phylogenetic analysis of the enterovirus recombinant strains. Phylogenetic trees were computed using sequences (**a**) cut before the putative recombination point [corresponding to nucleotides (nt) 518–833 of the CVA9 genome; GenBank D00627] or (**b**) after recombination (corresponding to nt 824–1,135 of the CVA9 genome). Two samples were collected from patient 2, and one sample was collected from patient 4. As references, corresponding sequences from representatives of Coxsackievirus A9 (*CVA9*), Echovirus 11 (*E11*) and Enterovirus 75 (*EV75*) were included. Coxsackievirus B1 (*CVB1*) was used as an outgroup. The NCBI accession numbers are shown in the tree

mononuclear cells). Enterovirus was determined by RT-PCR in the CSF and by cell culture in the stool, whereas bacterial cultures remained negative. He recovered rapidly and could leave the hospital after a short time.

Case 5

Ten days after case 1, the 36-year-old children's mother developed a similar condition. For 2 weeks she suffered from headache, which initially was strong and associated with fever and nausea. She was not admitted to hospital and no further investigations were conducted.

Results of molecular typing

Enterovirus strains sequenced from the CSF and swab samples from patients revealed no difference along the 618 bases analyzed [from nucleotide (nt) 518 to 1,135] with reference to human Coxsackie virus A9 (CV-A9) strain (Genbank accession D00627.1). Blast analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi) revealed that the first half of the sequence (from nt 518 to 833, with reference to D00627) was 88 % homologous to Enterovirus 75 (EV-75) (AY556070), 87 % homologous with Echovirus 11 (E-11) (AJ577594) strain, and 84 % homologous to CV-A9; in contrast, the second half of the sequence (from nt 833 to 1,135) was 77 % homologous to EV-75, 75 % homologous to E-11, and 91 % homologous to CV-A9. These results were further confirmed by a comparison of the phylogenetic trees obtained with fragments nt 518–833 and nt 834–1,135 from the sequences of the clinical strains with the CV-A9, EV-75, and E-11 strains (Fig. 1a, b). Therefore, we conclude that this strain is a recombinant strain with a 5′ untranslated region (UTR) and around 89 nucleotides of VP4 sequence originating from E-11/EV-75 and the rest of the genome originating from CV-A9 virus.

Discussion

Epidemic clusters of aseptic meningitis are caused mostly by Coxsackie B viruses and echoviruses. Coxsackie A9 viruses have also been associated with large outbreaks of aseptic meningitis [15–20]. This latter virus strain was found to be the causative agent in our family. Fever, severe headache, nausea, vomiting, and redness of throat have been reported in similar outbreaks and also observed in four of our family members [18, 19].

Transmission occurs efficiently by the fecal–oral route [3]. As such, the most likely route of transmission was from the 16-month-old child to his father and uncle, who both were regularly changing the infant's diapers. The other child was probably infected through direct contact with his index brother, whereas the mother was infected

secondarily by either her children or her husband. However, this chain of transmission cannot be proven due to lack of a positive RT-PCR results from both the youngest boy and the mother.

Recombination among different enterovirus strains co-circulating in the population is a well-known phenomenon [2, 21, 22]. Results from previous investigations on the molecular epidemiology of Coxsackie virus A9 suggest recombination predominantly in the nonstructural protein genome region [2], with hot spots of recombination having been mapped in enteroviruses in the 5′UTR and nonstructural genes [23].

The virus identified in this study is a recombinant Coxsackie virus A9 strain that acquired the 5′UTR and part of the VP4 coding sequence from an echovirus (likely E-11 or EV-75). To our knowledge, recombination breakpoints in capsid-encoding regions have not been described in the literature to date. The minor recombination in the capsid VP4 coding sequence did not change the antigenic properties of the maternal Coxsackie virus since the recombinant reacted with a monoclonal anti-Coxsackie A9 antibody but failed to react with a monoclonal anti-ECHO 11 antibody. The 5′UTR contains important secondary structures involved in the regulation of both replication and translation [24]. Therefore, acquisition of a new 5′UTR may improve virus fitness and in the cases reported in this study, virulence and contagiosity.

The rapid spread of this viral strain across Switzerland supports this hypothesis and the interpretation of genetic recombination as a driving force in the evolution of novel enterovirus lineages. A similar outbreak was observed 2 weeks later in a second geographic area roughly 100 km distant to first one, with the same virus being confirmed by phenotypical criteria. Another 2 months later this virus was shown to circulate in the western part of Switzerland some 250 km distant, presenting 97.9 % identity at the nucleotide level and 100 % identity at the amino acid level (C. Tapparel, personal communication).

Finally this recombination occurred between two members of the same human enterovirus B (HEV-B) species. To our knowledge, in vivo interspecies recombination has not been previously described.

Conflict of interest None.

References

1. Nasri D, Bouslama L, Pillet S, et al. Basic rationale, current methods and future directions for molecular typing of human enterovirus. Expert Rev Mol Diagn. 2007;7:419–34.
2. Santti J, Harvala H, Kinnunen L, et al. Molecular epidemiology and evolution of coxsackievirus A9. J Gen Virol. 2000;81:1361–72.
3. Horstmann DM. Enterovirus Infections: etiologic Epidemiologic And Clinical Aspects. Calif Med. 1965;103:1–8.
4. Hirst GK. Genetic recombination with Newcastle disease virus, polioviruses, and influenza. Cold Spring Harb Symp Quant Biol. 1962;27:303–9.
5. Ledinko N. Genetic recombination with poliovirus type 1. Studies of crosses between a normal horse serum-resistant mutant and several guanidine-resistant mutants of the same strain. Virology. 1963;20:107–19.
6. Oprisan G, Combiescu M, Guillot S, et al. Natural genetic recombination between co-circulating heterotypic enteroviruses. J Gen Virol. 2002;83:2193–200.
7. Lukashev AN, Lashkevich VA, Ivanova OE, et al. Recombination in circulating enteroviruses. J Virol. 2003;77:10423–31.
8. Jegouic S, Joffret ML, Blanchard C, et al. Recombination between polioviruses and co-circulating Coxsackie A viruses: role in the emergence of pathogenic vaccine-derived polioviruses. PLoS Pathog. 2009;5:e1000412.
9. Bailly JL, Mirand A, Henquell C, et al. Repeated genomic transfers from echovirus 30 to echovirus 6 lineages indicate co-divergence between co-circulating populations of the two human enterovirus serotypes. Infect Genet Evol. 2011;11:276–89.
10. Kyriakopoulou Z, Dedepsidis E, Pliaka V, et al. Full-genome sequence analysis of a multirecombinant echovirus 3 strain isolated from sewage in Greece. J Clin Microbiol. 2010;48:1513–9.
11. Santti J, Hyypia T, Kinnunen L, et al. Evidence of recombination among enteroviruses. J Virol. 1999;73:8741–9.
12. Kyriakopoulou Z, Dedepsidis E, Pliaka V, et al. Molecular identification and full genome analysis of an echovirus 7 strain isolated from the environment in Greece. Virus Genes. 2010;40:183–92.
13. Tapparel C, Cordey S, Van Belle S, et al. New molecular detection tools adapted to emerging rhinoviruses and enteroviruses. J Clin Microbiol. 2009;47:1742–9.
14. Guindon S, Gascuel O. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol. 2003;52:696–704.
15. Bello M, Mas Lago P, Palomera R, et al. Enterovirus meningoencephalitis in the last 5 years. Rev Cubana Med Trop. 1996;48:118–22.
16. Holowka A, Gawron H, Janowicz M, et al. An epidemic of meningitis in the Kwidzyn County caused by Coxsackie A9 virus. Przegl Epidemiol. 1975;29:433–7.
17. Kocielska W, Gruszecka A. An epidemic of meningitis caused by Coxsackie A9 virus in the city of Wroclaw in 1974. Przegl Epidemiol. 1975;29:431–2.
18. Connolly JH, O'Neill HJ. Coxsackie A9 virus outbreak in Northern Ireland during 1970. Ulster Med J. 1971;40:146–50.
19. Nakao T, Nitta T, Miura R, et al. Clinical and epidemiological studies on an outbreak of aseptic meningitis caused by coxsackie B5 and A9 viruses in Aomori in 1961. Tohoku J Exp Med. 1964;83:94–102.
20. Hinuma Y, Murai Y, Fukuda M, et al. An outbreak of aseptic meningitis associated with coxsackie B5 and A9 viruses in Northern Japan,1961. Virological and serological studies. J Hyg (Lond). 1964;62:159–70.
21. Oberste MS, Maher K, Pallansch MA. Evidence for frequent recombination within species human enterovirus B based on complete genomic sequences of all thirty-seven serotypes. J Virol. 2004;78:855–67.
22. Lukashev AN. Role of recombination in evolution of enteroviruses. Rev Med Virol. 2005;15:157–67.
23. Simmonds P, Welch J. Frequency and dynamics of recombination within different species of human enteroviruses. J Virol. 2006;80:483–93.
24. Fernandez-Miragall O, Lopez de Quinto S, Martinez-Salas E. Relevance of RNA structure for the activity of picornavirus IRES elements. Virus Res. 2009;139:172–82.