Genome Sequences of Rare Human Enterovirus Genotypes Recovered from Clinical Respiratory Samples in Bern, Switzerland

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ABSTRACT
We report on genomic sequences of human enteroviruses (EVs) that were identified in respiratory samples in Bern, Switzerland, in 2018 and 2019. Besides providing sequences for coxsackievirus A2, echovirus 11, and echovirus 30, we determined the sequences of rare EV-D68 and EV-C105 genotypes circulating in Switzerland. The viral genus Enterovirus belongs to the family Picornaviridae, which is associated with several human diseases (1). Some genotypes are predominantly isolated from respiratory samples (2, 3), e.g., the recently discovered species C genotypes enterovirus-C104 (EV-C104), EV-C105, and EV-C117 (4) or the well-known genotype EV-D68, which was linked to outbreaks of severe respiratory illness in children in summer 2014 in North America and cases of acute flaccid paralysis (5, 6). Various outbreaks of EV-D68 have since been reported worldwide (7–9).

A total of 145 respiratory samples from patients (average age of 3.5 years) who previously tested positive for EV or rhinovirus in 2018 or 2019 were screened for EV presence by PCR (ARGENE Rhino&EV/Cc R-GENE; bioMérieux, Geneva, Switzerland), immunofluorescence, and Sanger sequencing, as described previously (10, 11). Ethics approval was granted by the Swiss Ethics Committee (BASEC number Req-2018-00158). EVs were identified in six patients (4.1%), with two cases of EV-C105 and single cases of coxsackievirus A2 (CV-A2), echovirus 11, echovirus 30, and EV-D68. For one EV-C105 strain, EV-D68, and CV-A2, we further performed shotgun metatranscriptomic sequencing. Briefly, total RNA was extracted with the QIAamp viral RNA minikit according to the manufacturer’s instructions (Qiagen, Switzerland). Next, a 20-µL cDNA synthesis reaction was prepared. First, a mixture of 1 µL random hexamers (Thermo Fisher Scientific), 1 µL 10 mM deoxynucleoside triphosphate (dNTP) mix (New England Biolabs, Ipswich, MA, USA), 1 µL nuclease-free water (NFW), and 8 µL RNA extract was heated at 65°C for 5 min. Second, a preparation of 4 µL 5× SuperScript IV reaction buffer (Invitrogen, Carlsbad, CA), 2 µL dithiothreitol (DTT), 200 units SuperScript IV reverse transcriptase (Invitrogen), 40 units RNaseOUT (Invitrogen), and 1 µL NFW was made, and the two preparations were mixed and incubated at 25°C for 10 min, at 50°C for 10 min, and at 80°C for 10 min. The Illumina Nextera DNA Flex kit (paired-end reads, 300 cycles) was used for library preparation, followed by sequencing on a MiSeq benchtop sequencer, as described previously (12). Read trimming and quality filtering (fastp v0.20.0 [13]) were followed by genome assembly (SPAdes v3.13.2 [14]) and multiple sequence alignments (MAFFT v7.313 [15]). All software and scripts were used with default values. We thus recovered 96.7% of the genome sequence of EVCG-05-BE (EV-C105), 99.7% of EVCG-06-BE (EV-D68), and 85.7% of EVCG-03-BE (CV-A2), compared to their closest reference sequences (Table 1).

Sequence analysis showed that the Bern EV-D68 case clustered with subclade B3 sequences isolated from the United States (Fig. 1A), one of the most commonly reported EV-68 clades circulating worldwide in 2018 (16, 17). Analysis of all EV-C105 sequences...
### TABLE 1 Sample and sequencing details

| Sequence name | Taxonomy       | Collection date | Patient age (yr) | Type of sequencing | Sequence length (nucleotides) | GenBank accession no. | SRA accession no. | GenBank accession no. for closest sequence | Identity to closest sequence (%) |
|---------------|----------------|-----------------|------------------|--------------------|-------------------------------|-----------------------|-------------------|------------------------------------------|-------------------------------|
| EVCG-01-BE    | Echovirus E11  | December 2018   | <1               | VP1                | 322                           | OW121671              | MN121654.1        |                                          | 312/322 (96.9)               |
| EVCG-02-BE    | Echovirus E30  | December 2018   | <1               | VP1                | 325                           | OW122598              | MK815529.1        |                                          | 325/325 (100)                 |
| EVCG-03-BE    | CV-A2          | June 2018       | <1               | MTT                | 7,372                         | OW122596              | ERR9235688        | MT350223.1                                | 6,271/7,315 (85.7)            |
| EVCG-04-BE    | EV-C105        | February 2019   | 70–75            | VP1                | 275                           | OW122599              | KX901639.1        |                                          | 267/275 (97.1)                |
| EVCG-05-BE    | EV-C105        | March 2018      | <5               | MTT                | 7,288                         | OW122597              | KX276189.1        |                                          | 6,971/7,208 (96.7)            |
| EVCG-06-BE    | EV-D68         | September 2018  | <1               | MTT                | 6,898                         | OW122595              | MK419059.1        |                                          | 6,881/6,898 (99.7)            |

aVP1 gene amplicons were sequenced via Sanger sequencing; genome sequences were obtained by read assembly after shotgun metatranscriptomic (MTT) sequencing.
bThe closest sequence was that associated with the highest score via the NCBI BLASTn algorithm.
cFrom Illumina MiSeq paired-end sequencing (300 cycles). NA, not applicable.
(VP1) in GenBank showed that the two identified Bern EV-C105 isolates may correspond to different circulating strains (Fig. 1B). Only a few genome sequences of this globally circulating genotype (4, 18–20) are available to date (Fig. 1B).

Here, we report the first near-complete sequence of the EV-D68 genotype in Bern,
Switzerland. Outbreaks of EV-D68 were reported widely in Europe in 2018 (16, 21, 22). Together with six published sequences reported in 2018 in Basel, Switzerland (17), our study sheds light on the recent circulation of EV-D68 in Switzerland, a country with few data on this genotype to date. Furthermore, we present the first two sequences of the rarely reported EV-C105 genotype in Switzerland.

Data availability. The consensus genome sequences and associated raw data were deposited in the European Nucleotide Archive (ENA) under BioProject accession number PRJEB51320; the corresponding accession numbers for samples are provided in Table 1.

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REFERENCES

1. World Health Organization. 2015. Enterovirus surveillance guidelines: guidelines for enterovirus surveillance in support of the Polio Eradication Initiative. World Health Organization Regional Office for Europe, Copenhagen, Denmark. http://www.euro.who.int/__data/assets/pdf_file/0020/272810/EnterovirusSurveillanceGuidelines.pdf.
2. Royston L, Tapparel C. 2016. Rhinoviruses and respiratory enteroviruses: not as simple as ABC. Virus Res 8:16. https://doi.org/10.3390/v8010016.
3. Tapparel C, Siegrist F, Petty TJ, Kaiser L. 2013. Picornavirus and enterovirus diversity with associated human diseases. Infect Genet Evol 14:282–293. https://doi.org/10.1016/j.meegid.2012.10.016.
4. van Leer-Buter CC, Poelman R, Borger R, Niesters HGM. 2016. Newly identified enterovirus C genotypes, identified in the Netherlands through routine sequencing of all enteroviruses detected in clinical materials from 2008 to 2015. J Clin Microbiol 54:2306–2314. https://doi.org/10.1128/JCM.00207–16.
5. Midgley CM, Jackson MA, Selvarangan R, Turabelidze G, Obreinger E, Johnson D, Giles BL, Patel A, Echols F, Oberste MS, Watson JT, Gerber SI. 2014. Severe respiratory illness associated with enterovirus D68: Missouri and Illinois. 2014. MMWR Morb Mortal Wkly Rep 63:798–799.
6. Seijar JJ, Lopez AS, Cortese MM, Leshem E, Pastula DM, Miller L, Glaser C, Kambhampati A, Shioda K, Alilabadi N, Fischer M, Gregorius N, Lanciotti R, Nin WA, Sakthivel SK, Schmid DS, Oberste MS, Pallansch M, Feikin D. 2016. Acute flaccid myelitis in the United States, August–December 2014: results of nationwide surveillance. Clin Infect Dis 63:737–745. https://doi.org/10.1093/cid/ciw372.
7. Poelman R, Schuffenecker I, Van Leer-Buter C, Josset L, Niesters HGM, Lina B, ESCV-ECDC EV-D68 Study Group. 2015. European surveillance for enterovirus D68 during the emerging North-American outbreak in 2014. J Clin Virol 71:1–9. https://doi.org/10.1016/j.jcv.2015.07.296.
8. Funakoshi Y, Ito K, Morino S, Kinoshita K, Morikawa Y, Kono T, Aoda Y, Shimizu H, Hanaoka N, Konagaya M, Fujimoto T, Suzuki A, Chiba T, Akiba T, Tomanu Y, Watanabe K, Shimizu N, Horikoshi Y. 2019. Enterovirus D68 respiratory infection in a children’s hospital in Japan in 2015. Pediatr Int 61:768–776. https://doi.org/10.1111/ped.13903.
9. Holm-Hansen CC, Midgley SE, Fischer TK. 2016. Global emergence of enterovirus D68: a systematic review. Lancet Infect Dis 16:e64–e75. https://doi.org/10.1016/S1473-3099(15)00543-5.
10. Grädel C, Terrazos Miani MA, Barbaní MT, Leib SL, Suter-Riniker F, Ramette A. 2019. Rapid and cost-efficient enterovirus genotyping from clinical samples using Flongle flow cells. Genes (Basel) 10:659. https://doi.org/10.3390/genes10090659.
11. Barbaní MT, Gorgievski-Hrisohó M. 2009. Rapid detection of respiratory picornaviruses in nasopharyngeal aspirates by immunofluorescence assay. J Clin Virol 45:245–248. https://doi.org/10.1016/j.jcv.2009.05.008.
12. Grädel C, Terrazos Miani MA, Baumann C, Barbaní MT, Neunischwander S, Leib SL, Suter-Riniker F, Ramette A. 2020. Whole-genome sequencing of human enteroviruses from clinical samples by Nanopore direct RNA sequencing. Viruses 12:841. https://doi.org/10.3390/v12080841.
13. Chen S, Zhou Y, Chen Y, Gu J. 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34:i884–i889.
14. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Psham S, Prijibilski AD, Pyskin AV, Sirokin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.
15. Katoth K, Misawa K, Kuma-K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res 30:3059–3066. https://doi.org/10.1093/nar/guf436.
16. Bal A, Sabatier M, Wirth T, Coste-Burel M, Laznek M, Stefc K, Brengel-Pesce K, Morfin F, Lina B, Schuffenecker I, Josset L. 2019. Emergence of enterovirus D68 clade D1, France, August to November 2018. Euro Surveill 24:1800699. https://doi.org/10.2807/1560-7917.ES.2019.24.18.1800699.
17. Hodcroft EB, Dyrdak R, Andréès C, Egli A, Reist J, García Martinez de Artola D, Alcoba-Flórez J, Niesters HGM, Antón A, Poelman R, Reynolds M, Wollants E, Neher RA, Albert J. 2022. Evolution, geographic spreading, and demographic distribution of enterovirus D68. PLoS Pathog 18:e1010515. https://doi.org/10.1371/journal.ppat.1010515.
18. Tapparel C, Junier T, Gerlach D, Van-Belle S, Turin L, Cordey S, Mühlmann K, Regamey N, Aubert J-D, Soccol PM, Eigenmann P, Zdobnov E, Kaiser L. 2009. New respiratory enterovirus and recombinant rhinoviruses among circulating picornaviruses. Emerg Infect Dis 15:719–726. https://doi.org/10.3202/j1505081286.
19. Lukashev AN, Drexler JF, Kotova V, Regamey N, Aubert J-D, Soccol PM, Eigenmann P, Zdobnov E, Kaiser L. 2009. New respiratory enterovirus and recombinant rhinoviruses among circulating picornaviruses. Emerg Infect Dis 15:721–726. https://doi.org/10.3202/j1505081286.
20. Daleno C, Piralla A, Scala A, Baldanti F, Usonis V, Principi N, Esposito S. 2012. Complete genome sequence of a novel human enterovirus C (HEV-C117) identified in a child with community-acquired pneumonia. J Virol 86:10888–10889. https://doi.org/10.1128/JVI.01721-12.
21. Pellegrinelli L, Giardina F, Lunghi G, Uceda Renteria SC, Greco L, Fratini A, Pellegrinelli L, Giardina F, Lunghi G, Uceda Renteria SC, Greco L, Fratini A. 2020. Whole-genome sequencing of the consensus genome sequence of a novel human enterovirus C (HEV-C117) identified in a child with community-acquired pneumonia. J Virol 86:10888–10889. https://doi.org/10.1128/JVI.01721-12.
22. Cottrell S, Moore C, Perry M, Hilleves E, Williams C, Shankar AG. 2018. Prospective enterovirus D68 (EV-D68) surveillance from September 2015 to November 2018 indicates a current wave of activity in Wales. Euro Surveill 23:1800578. https://doi.org/10.2807/1560-7917.ES.2018.23.46.1800578.
23. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Biol Evol 35:1547–1549. https://doi.org/10.1093/molbev/msy096.