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[a]	Sequence length (nucleotides)	GenBank accession no.[b]	SRA accession no.	GenBank accession no. for closest sequence[b]	Identity to closest sequence (no. of identical nucleotides/total no. of nucleotides [%])[b]	Total no. of reads[c]	No. of reads mapping to EV (% of total)
EVCG-01-BE	Echovirus E11	December 2018	<1	VP1	322	OW121671		MN121654.1	312/322 (96.9)	NA	NA
EVCG-02-BE	Echovirus E30	December 2018	<1	VP1	325	OW122598		MK815529.1	325/325 (100)	NA	NA
EVCG-03-BE	CV-A2	June 2018	<1	MTT	7,372	OW122596	ERR9235688	MT350223.1	6,271/7,315 (85.7)	4,560,107	10,681 (0.23)
EVCG-04-BE	EV-C105	February 2019	70–75	VP1	275	OW122599		KX901639.1	267/275 (97.1)	NA	NA
EVCG-05-BE	EV-C105	March 2018	<5	MTT	7,288	OW122597	ERR9235689	KX276189.1	6,971/7,208 (96.7)	3,917,728	502 (0.013)
EVCG-06-BE	EV-D68	September 2018	<1	MTT	6,898	OW122595	ERR9235690	MK419059.1	6,881/6,898 (99.7)	2,526,310	1,360 (0.054)

[a]VP1 gene amplicons were sequenced via Sanger sequencing; genome sequences were obtained by read assembly after shotgun metatranscriptomic (MTT) sequencing.
[b]The closest sequence was that associated with the highest score via the NCBI BLASTn algorithm.
[c]From Illumina MiSeq paired-end sequencing (300 cycles). NA, not applicable.
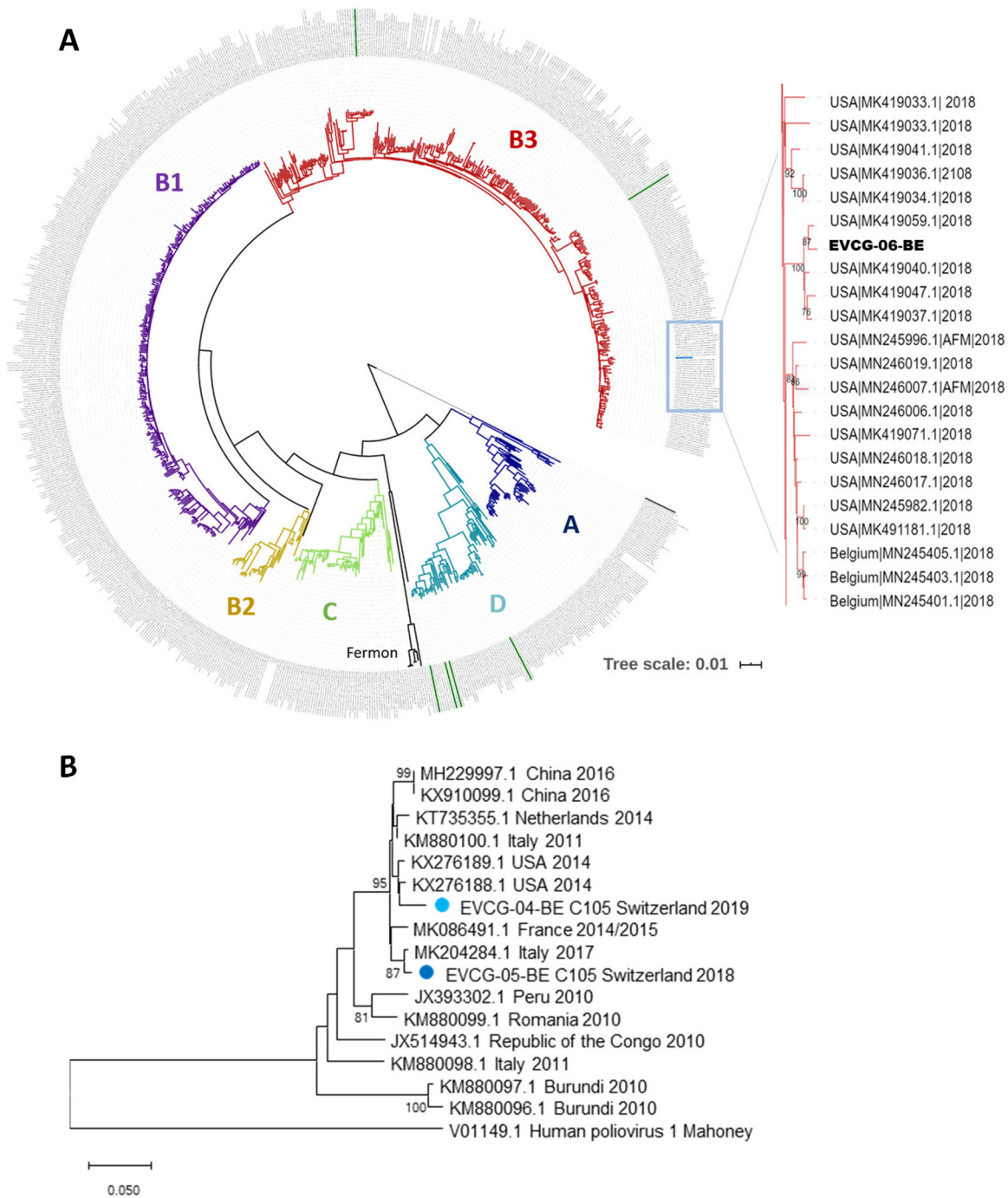
FIG 1 (A) Phylogenetic tree of the EV-D68 genome sequence (EVCG-06-BE) from Bern, Switzerland, with all available EV-D68 genome sequences. The inset on the right indicates phylogenetic relationships in the close neighborhood of the reported EV-D68 sequence (bootstrap support cutoff, ≥70%). Labels consist of isolate's country of origin, accession number, followed by year of isolation separated by vertical bars. Labels of other Swiss EV-D68 genomes retrieved from GenBank (MN245406 to MN245411) are colored in dark green in the circular phylogenetic tree. (B) VP1-based neighbor-joining phylogenetic tree of EV-C105 sequences. The two strains found in Bern, Switzerland (blue circles), were compared with all available EV-C105 sequences in GenBank on the basis of their partial VP1 sequences. Poliovirus 1 was added as an outgroup. Labels consist of sequence accession number, isolate's country of origin, followed by year of isolation separated by spaces. The scale bars represent the number of base substitutions per site. Bootstrap values were set to 1,000 replicates for both phylogenies. Phylogenetic trees were created using MEGA X (23) and further edited using the iTOL platform (https://itol.embl.de).

(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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