Epidemiology of Enterovirus Types Causing Neurological Disease in Austria 1999–2007: Detection of Clusters of Echovirus 30 and Enterovirus 71 and Analysis of Prevalent Genotypes

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Between 1999 and 2007 1,388 stool specimens from patients with acute flaccid paralysis or aseptic meningitis were submitted to the Austrian reference laboratory for poliomyelitis. Samples (201) yielded non-poliovirus enterovirus in culture. One hundred eighty-one viruses were available for typing and 78 isolates which remained serologically untyped were further analyzed by CODEHOP-PCR and sequencing of the VP1 gene and the 5′-untranslated region (5′-UTR). Typing revealed an Echovirus 30 outbreak in northwestern Austria in 2000, which was in accordance with the situation in Europe, and no dramatic seasonal changes of Coxsackie viruses were observed. In 2002/2003 a small outbreak of enterovirus 71 (EV71), affected 12 patients in the province of Styria. This virus was identified as genotype C1 and appeared to be genetically distinct from the isolates observed in 2001/2002 in Vienna. In 2004 two unrelated cases occurred in Lower Austria, which were identified as genotype C4, which has been described associated with high mortality most recently in China. In contrast to the situation in Asia the detected EV71 cases were not associated with hand–foot–mouth disease, but with serous meningitis only. This was surprising as a recent publication suggested a reduced neurovirulence of C1 genotype in children in Norway, presumably due to alterations in 5′-UTR and polymerase gene. However, comparing the 5′-UTR of the Austrian isolates and established virulent reference strains to the Norwegian isolate and an attenuated EV71 laboratory strain we did not find an indication that the genotype C1 possesses a RNA structure in its 5′-UTR leading to reduced neurovirulence. *J. Med. Virol. 81:317–324, 2009.*
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INTRODUCTION

Enteroviruses (EVs) are a leading cause of aseptic meningitis mostly affecting children in Europe in summer and fall season. Hand, foot, and mouth disease (HFMD) is frequently caused by Coxsackie virus-A type 16 (CA16) and enterovirus 71 (EV71). EV71 is considered the most dangerous EV after eradication of poliovirus in most parts of the world.

Findings in earlier outbreaks in Bulgaria and Hungaria have suggested an enhanced neurovirulence [Chumakov et al., 1979; Nagy et al., 1982] and most recently numerous deaths have occurred in China [WHO, 2008]. Lethality among young children due to EV71 have been observed in Malaysia (1997) and Taiwan (1998), whereas earlier outbreaks in Japan (1978) were large but rather benign [for review see Ho, 2000; McMinn, 2002; Lin et al., 2003]. This raises the question whether certain subtypes are associated with less severe disease [Chan and AbuBakar, 2005] or epidemiological and host factors are more important [Yang et al., 2001; Tseng et al., 2007]. Supporting the virulence hypothesis, a recent publication reported wide spread

unapparent infection with EV71 genotype C1 in Norwegian children [Witsø et al., 2007]. Data from Malaysia however, seem to contradict that as genotype C1 there had a higher rate of CNS infection and also differences in association to family clusters were observed among the genotypes C1, B4, and B5 [Ooi et al., 2007].

By molecular typing EV71 can be divided into three genogroups (A, B, C), and further sub-divided into genotypes B1-5 and C1-5 [Mizuta et al., 2005; Tu et al., 2007] which currently are co-circulating worldwide. Genotype C1 is dominating in Europe, but also found in Australia, Malaysia, and Singapore [Cardosa et al., 2003; Sanders et al., 2006]. In China, Taiwan and Japan other B and C genotypes are prevalent [Cardosa et al., 2003] obviously replacing each other in circulation every 1–2 years [Mizuta et al., 2005]. Whether these genotypes correlate to distinct serotypes leading to specific immunity is questionable, as recent data has provided evidence that antiserum against one genotype can neutralize the other genotypes of EV71 as well [Kung et al., 2007].

The WHO program for the eradicate poliomyelitis requires surveillance for EV in acute flaccid paralysis (AFP). Discrimination of EV species by serological methods only is insufficient and often hampered by non-neutralizeable virus due to aggregation [Nagy et al., 1982] or limited by the availability of suitable monospecific antisera for neutralization assays.

Molecular typing of EV is ideally conducted by DNA sequencing and the genotypes based on the VP1 capsid gene correlate well with serotypes determined by virus neutralization [Oberste et al., 1999]. Nevertheless PCR amplification of all EV types is still challenging due to genomic variability even within serotypes, which requires usage of multiple primers or highly degenerate primers. The difficulty to design "pan-EV" primers has been overcome by using the consensus degenerate hybrid oligonucleotide primer (CODEHOP) approach [Rose et al., 1998] and a semi-nested PCR protocol suitable for amplification of almost all EV and many human rhinoviruses [Nix et al., 2006].

Applying these methods the aim of this study was to identify the EV types causing neurological disease in Austria between 1999 and 2007 with a special focus on the epidemiology of EV71 isolates.

MATERIALS AND METHODS

Specimens, Culture, Serologically Typing

Clinical specimens (1,388) were submitted to the AGES poliovirus reference laboratory, Vienna, Austria between 1999 and 2007. Specimens (201) were found positive for EVs using several cell lines sensitive to poliovirus and other EVs. RD-Atlanta a human rhabdomyosarcoma cell, L20B a mouse L-cell line transfected with polio receptor, LLCMK2 and VERO, kidney cell lines from rhesus and African green monkey and the human larynx-epidermoid carcinoma cell line HEP-2 were used. The samples were treated with chloroform and antibiotics to eliminate bacteria and fungi. Virus isolation was performed by a conventional cell culture method as described elsewhere [WHO Polio Laboratory Manual, 2004]. Cultures were maintained at 36°C until cytopathic effect (CPE) was complete, and the infected cells harvested and stored at −20°C until typing.

Serotyping was done by virus neutralization using the typing sera from the RIVM (Bilthoven, The Netherlands) according to the manufacturer instructions. This kit contains reference typing horse antisera against the most frequently isolated human EV serotypes combined as nine antiserum pools. Virus controls and cell controls were run along for comparison. The plates were examined daily, until the virus control showed a 4 + CPE.

In 103 isolates the virus could be identified and the type assigned by the pattern of inhibition of CPE by antiserum pools. The remaining cultured isolates were reported as non-polio-enterovirus (NPEV) and further tested with molecular methods.

PCR Amplification, Molecular Typing

EV capsid gene sequences were amplified by a recently described semi nested PCR protocol leading to fragments of ~350–400 bp [Nix et al., 2006]. In brief, virus RNA was extracted from culture supernatants by silica based nucleic acid extraction (Viral Mini Kit, Qiagen, Hilden, Germany) and cDNA generated using four different primers (AN32-35). Five microliters of viral RNA were transcribed at 22°C for 10 min, and 42°C for 60 min in a total volume of 10 μl using Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA). Degenerate primers 222 and 224 were then used for first round amplification targeting the VP3 and VP1 region. Forty microliters of Platinum Taq polymerase reaction mix (Invitrogen) was added to the RT mix and a temperature profile of 95°C for 30 sec, 42°C for 30 sec, and 60°C for 45 sec was applied in a total of 40 cycles.

The internal primers AN88/89 used for the second round of amplification have been designed without Inosine residues using the CODEHOP strategy and target a conserved motif in the VP1 region [Nix et al., 2006]. One microliter of the product from the first PCR was added to 49 μl of reaction mix with above PCR reagents and amplification carried out at 95°C for 30 sec, 60°C for 20 sec, and 72°C for 15 sec in 40 cycles.

With the EV71 samples in addition to above regions extended length VP1 gene sequences were amplified using primers 159/162 and 189/011 leading to fragments of 485 and 797 bp respectively [Brown et al., 2000; Oberste et al., 2004]. Additionally also the 5′-UTR region was amplified in EV71 isolates by primers 294/295 and EVP2/OL68-1 leading to fragments of 645 and 750 bp [Oberste et al., 2004; Lin et al., 2006].

Genotype Analysis, Sequence Alignment, Statistical Analysis

Automated DNA sequencing of the amplified fragments was carried out on both strands and the result analyzed by the BLAST algorithm using the Genbank database.

Alignment with selected reference sequences obtained from Genbank was performed using the Clustal W algorithm in Sequencher, a DNA assembly software by Gene Codes Corp. (Ann Arbor, MI). Neighbor-joining trees were generated with MEGA using bootstrap analysis, and finally trees were plotted by Phylip 3.65.

Kimura two-parameter model of nucleotide substitution was used for calculation of the genetic distance of the compared DNA sequences using MEGA 3.1 software. Phylogenetic trees were constructed using the neighbor-joining method with 1,000 pseudoreplicate datasets. Bootstrap values representing percentage of pseudoreplicates are shown at the nodes.

Data of the 181 patients were collected and analyzed by using EpiInfo 3.2.2. (http://www.cdc.gov/epiinfo/). Analyzing the age distribution the interquartile range (IQR, difference between third and first quartile, 25–75%) was used because there was no normal distribution of the data. Additionally the age median of the children younger than 15 years (N = 165) was calculated separately (Fig. 1). For calculation of significance Fisher's Exact Test was applied.

RESULTS

Cases

The mean age of infection with EV was between 5 and 6 years and more than 90% of cases occurred before the age of 14 (Fig. 1). The mean age of EV71 case was calculated separately and was found to be 5.5 years (not shown).

A clear predominance of males was seen among EV cases (111 out of 181, 61.4%). Significantly (P = 0.0479) more boys were found in school children (5–14a) as compared to 0–4a old pre-school children (61/44) while the exact opposite was found in the girls group (26/34). The lead symptom was by far aseptic meningitis (65.6%)
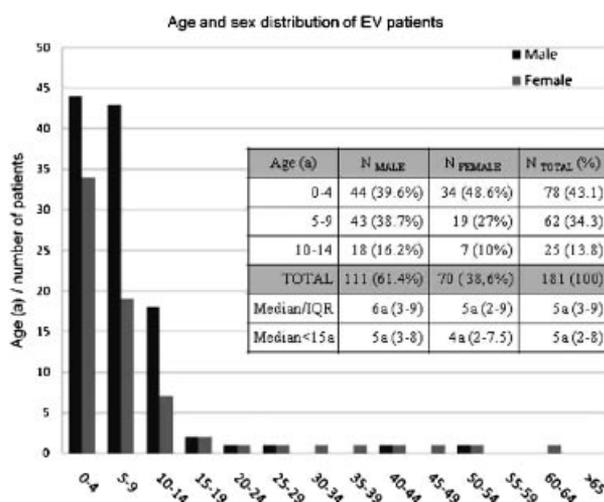

Age and sex distribution of EV patients

Age (a)	N MALE	N FEMALE	N TOTAL (%)
0-4	44 (39.6%)	34 (48.6%)	78 (43.1)
5-9	43 (38.7%)	19 (27%)	62 (34.3)
10-14	18 (16.2%)	7 (10%)	25 (13.8)
TOTAL	111 (61.4%)	70 (38.6%)	181 (100)
Median/IQR	6a (3-9)	5a (2-9)	5a (3-9)
Median<15a	5a (3-8)	4a (2-7.5)	5a (2-8)

Fig. 1. Distribution of age and sex of EV patients. Numbers of EV cases are listed in the different age groups. Note the significant (P = 0.0479) overrepresentation of males (dark gray bars) in the age group of 5–14a as compared to children younger than 4a. Median age and interquartile range (IQR) of the whole patient groups and cases younger than 15a are additionally shown.

followed by diarrhea (16.6%) or non-specific febrile illness (4.9%). Encephalitis and flaccid paralysis together accounted for another 7% whereas HFMD, caused by CA16, was only noted in two cases (1.2%) (Table I).

Due to an outbreak in 2000 in Upper Austria and Salzburg, Echovirus 30 (E30) was the most frequent virus causing aseptic meningitis followed by coxsackievirus B types 1–6 (CB1–6) and EV71 (Table II).

Distribution of EV Types in Cases of Infection With NPEV

As shown in Figure 2, out of 181 cases, infection with E30 accounted for 26.5% (48), with E6 and E13 for 5.5% (10), with E25 for 5% (9), with E18 and E11 each for 2.2% (4), with E9 for 1.7% (3), with E4 for 1.1% (2), and with E5, E7, E14, E15, E22, E29, E32, E90 each for 0.6% (1). Cases of infection with coxsackievirus B, which was not further sub-typed in most cases, accounted for 18.8% (34) and coxsackie A virus for 18.2 (33) including CA24 (24.4%), CA4 (9.1%), CA16 (15.2%), CA9 (15.2%), CA6 (15.2%), CA2 (3%), CA7 (3%), CA10 (3%), CA14 (3%). EV71 was found in 8.8% (16) of total cases. Distribution of clinical manifestation and causing EV type is given in Table I. In 2000, 31 cases of aseptic meningitis caused by infections with E30 clustered in 2 neighboring provinces of Austria, that is, Upper Austria and Salzburg. In the same year also a small outbreak of diarrhea among Austria, children (n = 7, median age 4 years) caused by CA24 occurred which was associated with stay in one hotel in Turkey (Fig. 3).

A cluster of 12 cases of aseptic meningitis caused by infection with EV71 was observed from 2002 to 2003 (Fig. 3) which seemed to be restricted to the province of Styria. Whether this is only due to the vigility of a special children's hospital or real limited to a certain area cannot be addressed for certainty, as this children's hospital traditionally was a leading institution for requesting EV culture in neurological cases, which is rarely done by most other hospitals.

There was a drop in EV isolation rate observed in the years 2006/2007 but we do not have an explanation for this, except that these 2 years appeared to be rather poor EV seasons in Austria (Table II).

Genotyping of EV71 Isolates

Based on 891 bp VP1 sequences the EV71 isolates (n = 14) from 2001 to 2003 grouped with the European C1 isolate from Norway and C1 isolates from Australia, Singapore and Sarawak, Malaysia. Interestingly, the EV71 isolates which occurred 2001 in the capital city of Vienna (#15,19) and the only isolate from early 2002 (#22) can be genetically separated from the isolates obtained from the 11 cases which occurred in the late 2002 and 2003 in Styria (#21, 27, 32, 33, 37, 40, 41, 42, 43, 44, 46). This was observed with both VP1 gene and 5′-UTR sequencing (Figs. 4 and 5) and indicates that a least two distinguishable sub-lineages of EV71 genotype C1 were present in different geographic locations within a small country like Austria.

TABLE I. EV Patient Case Features

Clinical manifestation	N (%)	Detected EV isolates
Aseptic meningitis	107 (65.6)	E30 (36.4%), CB1–6 (19.6%), EV71 (13.1%), E6, E13 (7.5%), E18, E25 (2.8%), E4, E5, E11 (0.9%)
Diarrhea	27 (16.6)	CA24 (25.9%), CA6, CB1–6, E30 (14.8%), CA2, CA4, EV90
Non-specific febrile illness	8 (4.9)	CB1–6 (25%), EV71 (12.5%), E18, E25, E39
Encephalitis	7 (4.3)	E11, E25, E30, CA4, CA7, CA24, CB1–6
Flaccid paralysis	4 (2.5)	E4, E7, CA16, CA24
Exanthem	2 (1.2)	E18, E25
Hand, foot, mouth disease (HFMD)	2 (1.2)	CA16 (100%)
Respiratory disease	2 (1.2)	E30, CA16
Abdominal pain	2 (1.2)	E25, E30
Hepatitis	1 (0.6)	E30
Myopericarditis	1 (0.6)	CB3

The Austrian EV71 genotype C4 isolates (#54, 69), which occurred in 2004, were homologous to Asian C4 strains from China, Taiwan and Japan (Fig. 4).

Also alignment using 5′-UTR sequences of the EV71 isolates (#15–46) showed genotype C1, whereas the isolates #54 and #69 grouped with Asian genotype C4 isolates causing the most recent outbreak in the Fujang Anhui province of China 2008 (Fig. 5).

Analysis of 5′-UTR Regions Possibly Involved in Neurovirulence

Further comparison of 5′-UTR sequences with available published sequences was performed to address the question of a potentially reduced neurovirulence caused by mutations in the 5′-UTR as proposed recently. Dr. Shimidzu's group in Japan has attenuated EV71 genogroup A prototype strain BrCr by inserting genomic mutations which mimic the difference between wild type poliovirus 1 Mahoney and the Sabine vaccine. They demonstrated that this isolate (EV71 S1-3′) has reduced neurovirulence in cynomolgus monkey [Arita et al., 2005].

We therefore looked if similar variations are present at the suggested positions also among the other EV71 genogroups and may relate to their pathogenesis and virulence. 5′-UTR sequences from positions 410 to 600 of C1 and C4 strains from this study and the Norwegian C1 isolate were aligned together with published sequences from C2, C3, C4, B4, and B5 prototype strains. We found that the nucleotide at position 485 of the 5-UTR region is an highly conserved Adenine in all the different genotypes of EV71, same as with BrCr strain or polio 1 wild-type. Thus A to G change in position 485 observed in the attenuated strain of EV71 was not seen in any other genogroup of EV71 including the presumably less virulent Norwegian C1 isolate. Similarly, the amino acid differences between EV71-BrCr and EV71 S1-3′ at 3D^{pol} position 73 (Tyr to His) and position 363 (Cys to Ile) were also not seen in the different genogroups of EV71. EV71-type A strain BrCr, C4, B4, and B5 all have Tyr whereas C1, C2, C3 and C5 have Phe at 3D^{pol} position 73. Furthermore all EV71 isolates analyzed in this study also have Cys at 3D^{pol} position 363 and show an Adenine also in the nucleotide position 7409, thus no A to G change was noticed as in the attenuated strain (comparisons not shown).

DISCUSSION

The main objective of this study was to investigate the distribution of EV types causing neurological diseases in Austria with a special emphasis on the emergence of EV71 genotypes and comparison to Asian genotypes.

More than 90% of EV cases were children under the age of 14 and a clear predilection of males was observed (61.4%). Additionally a significant higher proportion of boys was found in the group of the school children. Whether this indicates that EVs lead to a more severe course in males or school boys only might have an overall

TABLE II. Annual Distribution of NPEV Samples and Most Frequent Isolates

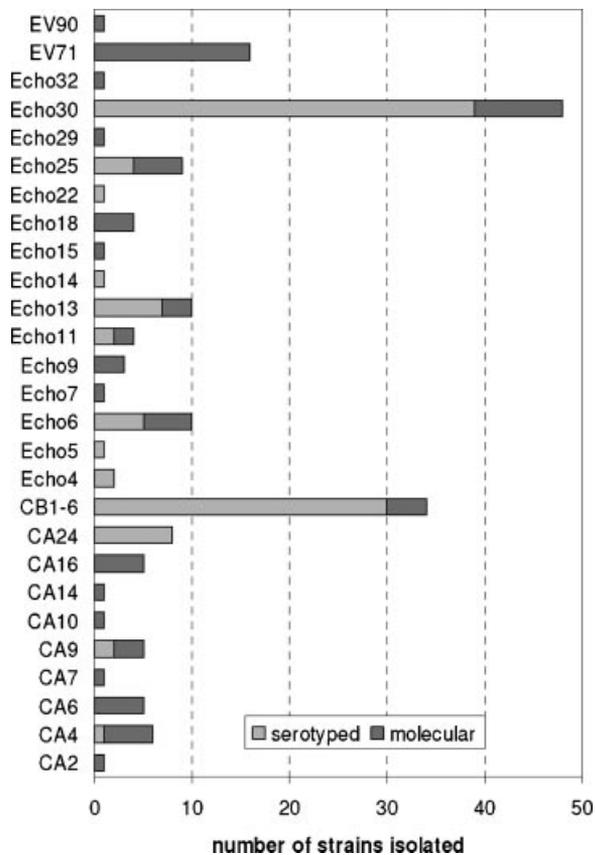| Year	Samples tested	NPEVs isolated	Isolation rate (%)	Serol. typed	Molec. typed	E30	CB1–6	EV71	E13	E6
1999	133	14	10.5	14	0	3	1	0	0	0
2000	210	57	27	50	7	31	9	0	7	6
2001	137	19	14	7	12	2	3	2	1	1
2002	152	32	21	11	21	6	5	6	2	2
2003	147	14	9.5	2	12	0	2	6	0	0
2004	155	19	12	8	11	1	8	2	0	0
2005	151	15	10	9	6	3	3	0	0	0
2006	152	4	2.6	1	3	0	2	0	0	0
2007	151	7	4.6	1	6	2	1	0	0	1
Total	1,388	181	13	103	78	48	34	16	10	10

Fig. 2. Distribution of EV strains assigned by serotyping and molecular typing. Coxsackie virus A (CA2–24), Coxsackie virus B (CB1–6), echovirus types (Echo 4–32) and enterovirus (EV) types 71 and 90 were found. Bars represent number of isolates with scale indicated at the x-axis.
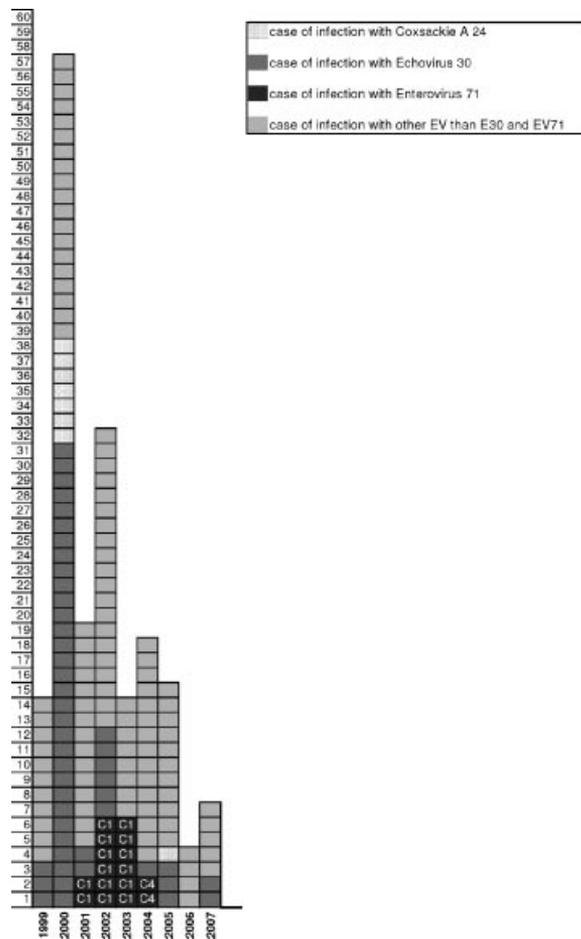

Fig. 3. Seasonal EV distribution and genotypes of Austrian EV71 isolates. Cases of infection with EV (n = 181) by year, are shown from 1999 to 2007. Cluster of cases of E30-infection in 2000 (n = 31) and cluster of EV71 infection (n = 14) from 2001 till 2003 in Austria. The genotype of the EV71 isolates as determined by sequencing is depicted within the columns.

higher infection rate than girls for some reasons (of cleanliness, etc.) remains unclear, as our study only covers clinical cases.

Echoviruses (n = 65) were the most frequent agent causing aseptic meningitis. E30 was the most frequent EV isolated (48 cases) from 1999 to 2007 with a peak incidence in 2000 (n = 31). E13 (7/10 isolates) and E6 (6/10 isolates) were also most frequently isolated in 2000 and restricted more or less to the Northern-East part of Austria. Enhanced isolation of echoviruses in 2000 was also observed in Spain, France, and Belgium [Chomel et al., 2003; Thoelen et al., 2003; Trallero et al., 2003]. In Germany E30 was the most frequent EV in 2000 and 2004/2005, and it has been proposed as an indicator for overall strong EV activity [Roth et al., 2007]. E30 was also the cause of an outbreak of aseptic meningitis in France 2005 with co-circulation of distinct sub-lineages [Brunel et al., 2008].

Despite a small outbreak of CA24 in 2000, which could be traced to a hotel in Turkey, the EV infections of our study group in most cases were not associated with a cluster in time and in place and no apparent annual changes were observed with both coxsackievirus groups.

CB1–6 accounted for 18.8% of NPEV isolates and CA viruses for 18.2%. It has to be noted that diarrhea was more frequently associated with CA than other EV species.

There was a cluster of infection with EV71 genotype C1 in 2002 and 2003, where all cases fell ill from aseptic meningitis (n = 12). This is of particular interest as EV71-C1 has been suggested in a recent study in Norwegian children to exhibit a reduced neurovirulence, and alterations in the 5′-UTR have been considered a possible cause [Witsø et al., 2007]. However, the 5′-UTR sequences of the neurovirulent Austrian EV71 isolates between 2001 and 2003 were found very similar to the Norwegian isolate from 2003.

Conclusive results on viral genomic variation among different genotypes related to their virulence are still lacking. The study of Witsø et al. indicated that the C1 genotype possesses a different predicted RNA structure on its 5′-UTR region which might lead to reduced neurovirulence. Their predicted RNA structure on the 5′-UTR
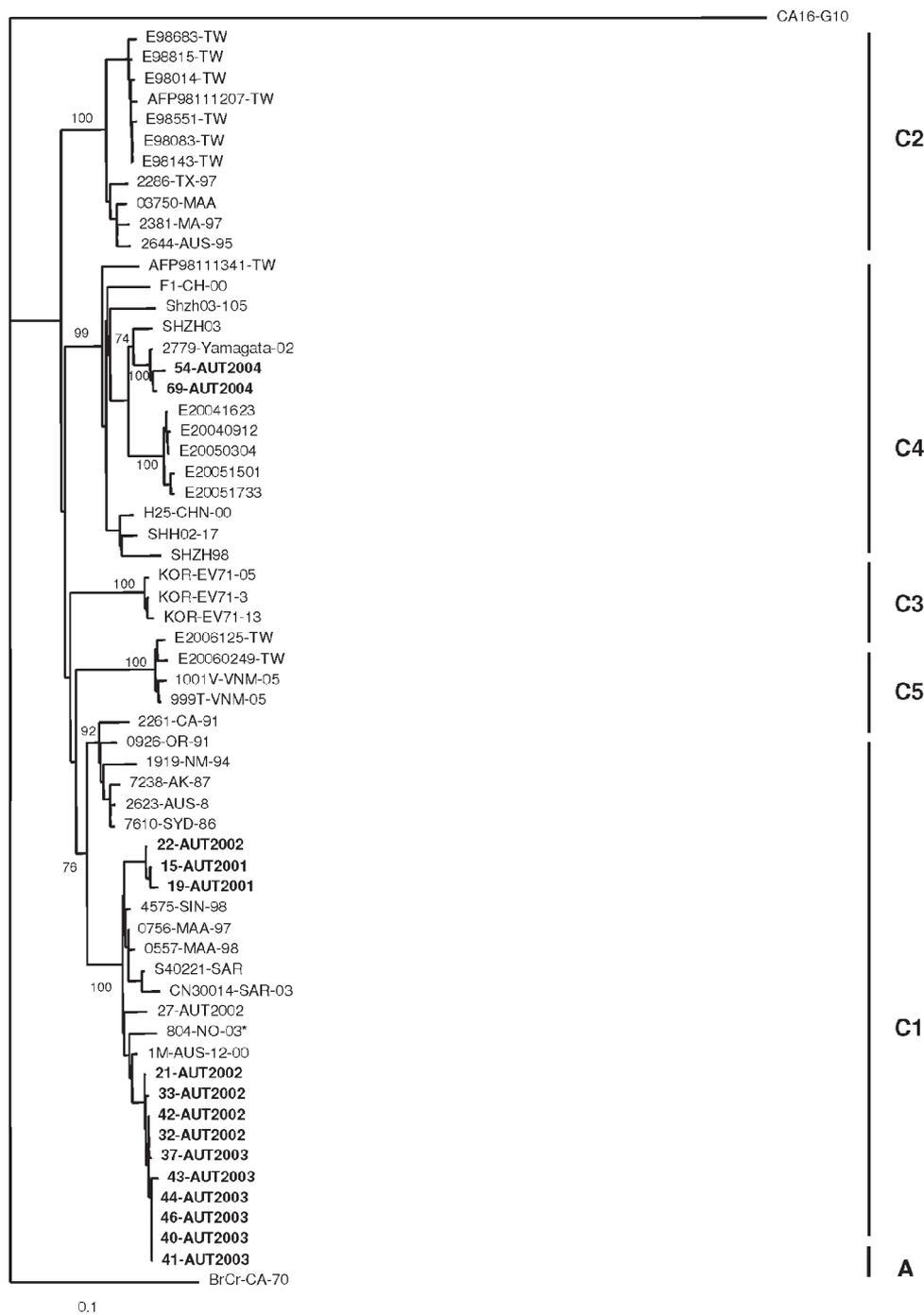
Fig. 4. Phylogenetic analysis of the 891 bp VP1 sequences with published EV71 reference strains. Branch lengths are proportional to the relative phylogenetic distance. Scale bar represents the genetic distance (nucleotide substitutions per site). Bootstrap probabilities as percentages of 1,000 pseudoreplicate datasets are indicated above the branches. Only EV71 genotype C and A strains are shown. Related Coxsackie virus A16 is depicted on the top for comparison. Asterisk indicates subtype C1 sequence found in asymptomatic children in Norway 2003. Austrian isolates are highlighted, note the phylogenetic separation of C1 isolates according to differences in time and regional location (#15,19 from capital city of Vienna and #21–41 from province of Styria). The two genotype C4 isolates originate from an 8-year-old male with Vietnamese origin living in Styria province (#54) and an 8 months female from Lower Austria (#69).

region based on the Zuker algorithm (version 3.71) is not the same as that of poliovirus proposed by Pilipenko et al. [1989] and results in different predicted domain V structures in the 5′-UTR stem-loop region. We also obtained different predicted structures when the MFOLD 3.1 program was used. It is therefore difficult to determine whether the predicted 5′-UTR stem-loop region reflects the in vivo situation. Further genomic construction experiments will be needed to address this question.
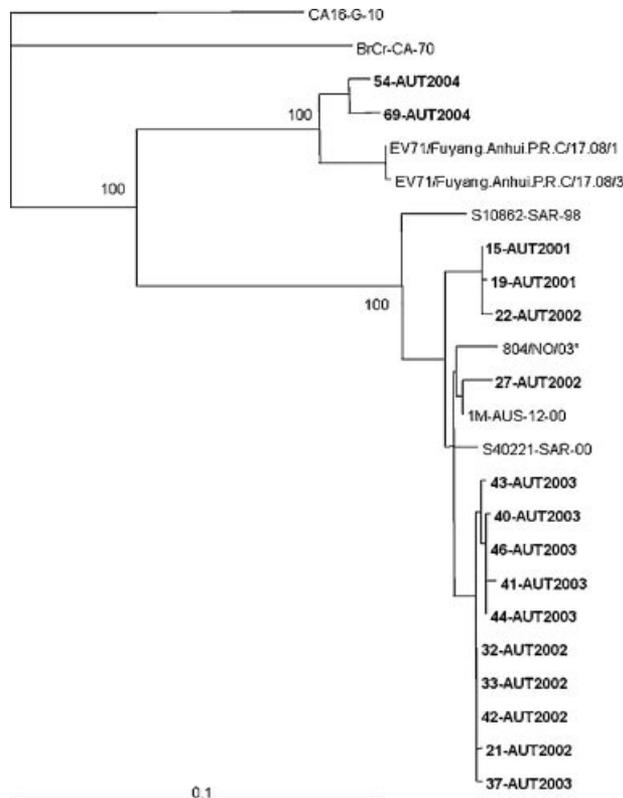
Fig. 5. Phylogenetic analysis of the 5-prime untranslated region (5′-UTR) sequences of Austrian EV71 isolates. Comparison with genotype C1 strains from Norway, Australia and Indonesia (Sarawak) is shown. EV71 genotype C4 isolates from Fuyang Anhui province of China 2008 are used as reference for the two Austrian C4 isolates from 2004. Coxsackievirus A16 (CA16), strain G10 was used as an outgroup in the analysis. Branch lengths are proportional to the relative phylogenetic distance. Scale bar represents the genetic distance (nucleotide substitutions per site). Alignment of the 723 base pairs of 5′-UTR sequence was undertaken by using the Clustal W program. A phylogenetic tree was constructed based on the neighbor-joining method with Phylip 3.65. The reliability of the neighbor-joining tree was estimated by bootstrap analysis with 1,000 pseudoreplicates.

All established genotypes of EV71 so far have been shown to cause severe and fatal infections, especially among very young children. The incidence of CNS disease has been variable between published EV71 outbreaks for unknown reasons, differences in virulence between genotypes or double infections (including non-EVs) have been suggested as possible reasons [Ooi et al., 2007]. This prospective study from Malaysia revealed that children with genotype B4 were less likely to get CNS complications than those with B5 or C1. These findings would conflict with the assumption of a less neurovirulent character of C1 subtypes. The Norwegian study collected stool samples from healthy children but not from symptomatic patients, it therefore is difficult to draw the general conclusion that because the virus was isolated from healthy children, strain 804-NO-03 would be apathogenic or less virulent than other genotypes. Vice versa the finding that all our EV71 samples originated from patients having neurological symptoms is what was expected as they were selected from the AFP program.

However, it certainly cannot be ruled out that there are indeed strain differences between European and Asian isolates even within the same genogroup. Thus in contrast to the Asian situation the Austrian EV71 samples were from patients which did not have any HFMD symptoms but serous meningitis only. Maybe this is a hint towards some strain differences of European C1 strains and the C1 isolates from Western Australia, Malaysia and Singapore, which have been reported primarily associated with HFMD [McMinn, 2002].

EV infection is seasonal in temperate climate whereas a year round incidence occurs in tropical and subtropical areas. Therefore the human EV activity is higher in Taiwan, Malaysia and Singapore than in Japan, Norway or Austria. Disregarding possible genetic differences in vulnerability of patients, climate differences alone may account for a different outcome. The much higher population densities in Asia and social factors of work and child bearing are expected to lead to a lower average age at infection and more severe symptoms consecutively than in aging European societies.

Introduction of new EV71 strains is a big concern and genotype C4 has been introduced from China to Taiwan recently [Lin et al., 2006]. The only two EV71 genotype C4 isolates we encountered in Austria in the year 2004 did not have any obvious connection to each other and originated from distinct areas. Although family anamnesis revealed no primary travel association, the fact that one of the children was of Vietnamese mother tongue suggests that independent introduction of Asian strains may occur in middle Europe.

Whether sporadic detection of Asian type C4 isolates would have a big threat potential remains questionable, despite of public concerns following alarming reports of numerous fatal cases by similar strains in China most recently [WHO, 2008]. The observed mean age of infection of more than 5 years among EV71 cases in Austrian children, together with a lower overall EV incidence certainly contribute to the fact that fatal cases were not observed in Austria so far.

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