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Molecular epidemiology of outbreaks of gastroenteritis associated with small round structured viruses in Germany in 1997/98

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Summary. The molecular epidemiology of small round structured viruses (SRSVs) in Germany was studied using fecal specimens from 16 SRSV-associated gastroenteritis outbreaks in different parts of Germany during 1997/1998 by reverse transcription-polymerase chain reaction (RT-PCR) and by sequencing of the ORF1 and ORF3 amplicons. The majority of the isolates clustered in one subtype and were closely related to published SRSV sequences of genogroup II.

Introduction

Small round structured viruses (SRSVs) are members of the genus Calicivirus within the family *Caliciviridae* which are associated with gastroenteritis in humans [2, 4, 14]. The course of the illness is usually mild and self-limiting, but the viruses are highly infectious. The prototype strain of human SRSVs is Norwalk virus which was first described in 1972 [13]. By electron microscopy SRSVs can be distinguished from classical human Caliciviruses (HuCVs) which have distinctive cup-shaped surface depressions. The SRSVs have a positive sense single-stranded RNA genome of approximately 7.5 kb which contains three major open reading frames (ORFs) [12, 16]. ORF1 encodes a large polyprotein including the RNA-dependent RNA polymerase motifs, whereas ORF2 and ORF3 encode a single structural capsid protein [11, 12] and a small, basic protein of unknown function, respectively.

The family *Caliciviridae* has been divided into three genogroups and SRSVs have been assigned to genogroups I and II. At present, viruses of genogroup II are divided into two subgroups [23]. The HuCVs form genogroup III [9, 22].

The identification and molecular characterization of SRSVs of 16 separate acute gastroenteritis outbreaks in different parts of Germany between 1997 and 1998 is described in detail here. Two RT-PCR primer sets were designed based on the nucleotide sequence diversity in the RNA polymerase gene (ORF1) and in the small, basic protein gene region (ORF3). The phylogenetic rela-

Table 1. SRSV-associated gastroenteritis outbreaks in Germany

Designation	Location of outbreak	Date (month/year)	Setting(s)	Cases of illness	No. of specimens in study
HSS/3/97/DEU	Hesse	3/97	nursing home	47	7
SA/1/98/DEU	Saxony-Anhalt	1/98	old people's home	66	7
BAV/1/98/DEU	Bavaria	1/98	old people's home	47	18
BAV/2.1/98/DEU	Bavaria	2/98	old people's home	93	2
BAV/2.2/98/DEU	Bavaria	2/98	hospital	61	7
SA/2/98/DEU	Saxony-Anhalt	2/98	sugar factory	39	4
BRA/2.1/98/DEU	Brandenburg	2/98	hospital	66	8
BRA/2.2/98/DEU	Brandenburg	2/98	old people's home	27	6
BRA/2.3/98/DEU	Brandenburg	2/98	old people's home	23	5
NRW/2/98/DEU	North Rhine Westphalia	2/98	old people's home	28	10
HSS/2/98/DEU	Hesse	2/98	rehabilitation center	34	2
BRA/3/98/DEU	Brandenburg	3/98	hospital	16	8
BLN/3/98/DEU	Berlin	3/98	hospital	8	5
RP/3/98/DEU	Rhineland-Palatinate	3/98	hospital	15	8
HSS/3/98/DEU	Hesse	3/98	rehabilitation center	79	2
SAX/4/98/DEU	Saxony	4/98	hospital	74	11

tionship of the SRSVs detected in this study with those previously reported is analyzed.

Materials and methods

Outbreaks

Fecal specimens from 16 gastroenteritis outbreaks suspected to be SRSV-associated were sent to Robert Koch-Institute for virological investigation. All stool samples were negative for Salmonella, Shigella, Campylobacter species as well as for Rota-, Adeno-, Astro- and Enteroviruses. More detailed information about the SRSV-positive outbreaks is given in Table 1.

Oligonucleotide primers

The primers used for cDNA synthesis and PCR amplification were for the ORF1 (primer position according to GenBank Acc. no. X86557): 32 (5′-^{4226} ATG AAT ATG AAT GAA GAT GG-3′); 33 (5′-^{4280} TAC CAC TAT GAT GCA GAT TA-3′); 35 (5′-^{4617} GTT GAC ACA ATC TCA TCA TC-3′); 36 (5′-^{4707} ATT GGT CCT TCT GTT TTG TC-3′) and for the ORF 3: 49 (5′-^{6686} GCG CAG GCG TGC ATT ATA ATG-3′); 51 (5′-^{6724} TGG ATT GGC ATC TGA TGT C-3′); 54 (5′-^{7035} CAG TCC AGA GCC TTT GTC AT-3′); 55 (5′-^{7069} CCT GGC ATC AGG GGC CCA GTA-3′).

RNA extraction and RT-PCR

RNA was extracted from 140 μl of the 10% fecal suspension by a spin column technique using the QIAamp viral RNA kit according to manufacturer's instructions (QIAGEN; Hilden,

Germany). One-tenth of the extracted RNA was reverse transcribed using M-MuLV-RT (Life Technologies, Gaithersburg, MD) and primers 36 for ORF1 and 55 for ORF3. Part of the cDNAs were amplified by nested PCR using primers 32/36 (ORF1) and 49/55 (ORF3) for the first round, and primers 33/35 (ORF1) and 51/54 (ORF3) for the second round PCR.

Amplifications were performed for 35 cycles (94 °C, 30 sec; 42 °C, 30 sec; 72 °C, 45 sec; for the last 3 min extension at 72 °C).

DNA sequencing and phylogenetic analysis

Second round PCR products of 338 bp for the ORF1 and of 312 bp for the ORF3, respectively, were sequenced directly in both directions with the second round PCR primers using an ABI Prism 377 DNA sequencer and DNA dye terminator cycle sequencing kit (Perkin-Elmer). Alignments of ORF1 (nts. 4616–4849) and ORF3 (nts. 7013–7243) were performed with the CLUSTAL W program version 1.6. The nucleotide positions (excluding the primer regions) corresponds with the Norwalk prototype sequence (GenBank Acc. no. M87661). Phylogenetic trees were produced using the Phylogeny Interference Package (PHYLIP) version 3.57c [5, 6]. Evolutionary distances were estimated using the DNADIST program, and unrooted phylogenetic trees were constructed using the neighbor-joining method (PHYLIP). The analysis of 1000 bootstrap resamples of the nucleotide alignment data sets was performed using the SEQBOOT and CONSENSE programs of PHYLIP.

Results

To investigate the genetic variability of SRSVs in Germany, we determined the nucleotide sequence of part of the putative RNA polymerase region (ORF1) and of part of the small basic protein (ORF3) for 16 outbreaks (November 1997 to May 1998) positive in the RT-PCR for SRSV. The majority of these outbreaks occurred in either old people's homes (37%) or hospitals (37%) (see Table 1).

RT-nested-PCR screening was performed with broadly designed reactive primer pairs in the ORF1 which amplify both genogroups I and II SRSV RNA in fecal material. Our primers created in the ORF3 should only be specific for genogroup II of SRSV sequences based on sequences published up to now. The nested PCR products were subjected to direct sequencing with primer pairs from which the amplicons were obtained.

Isolates from 15 of the 16 SRSV-associated outbreaks analyzed by this method in ORF1 and ORF3 were shown to belong to genogroup II and only one to genogroup I according to the published classification of the SRSV in two genetic groups. No sequence differences were found among specimens investigated within one outbreak.

However, an overall comparison of sequence data obtained for viruses from these 16 outbreaks and published sequence data regarding ORF1 and ORF3 suggests a subdivision of SRSVs into different genotypes and subtypes in a generated unrooted phylogenetic tree of the nucleotide and amino acid sequences (Fig. 1). According to this, isolates with nucleotide differences of $\geq$ 23% belong to different genotypes, isolates of the same subtype showed nucleotide differences of $\leq$ 12%. The designation of genotypes is arbitrary.

Of the 16 confirmed SRSV-associated outbreaks, 11 were shown to be caused by viruses with greater than 90% identity with Lordsdale/UK/93 or Camber-

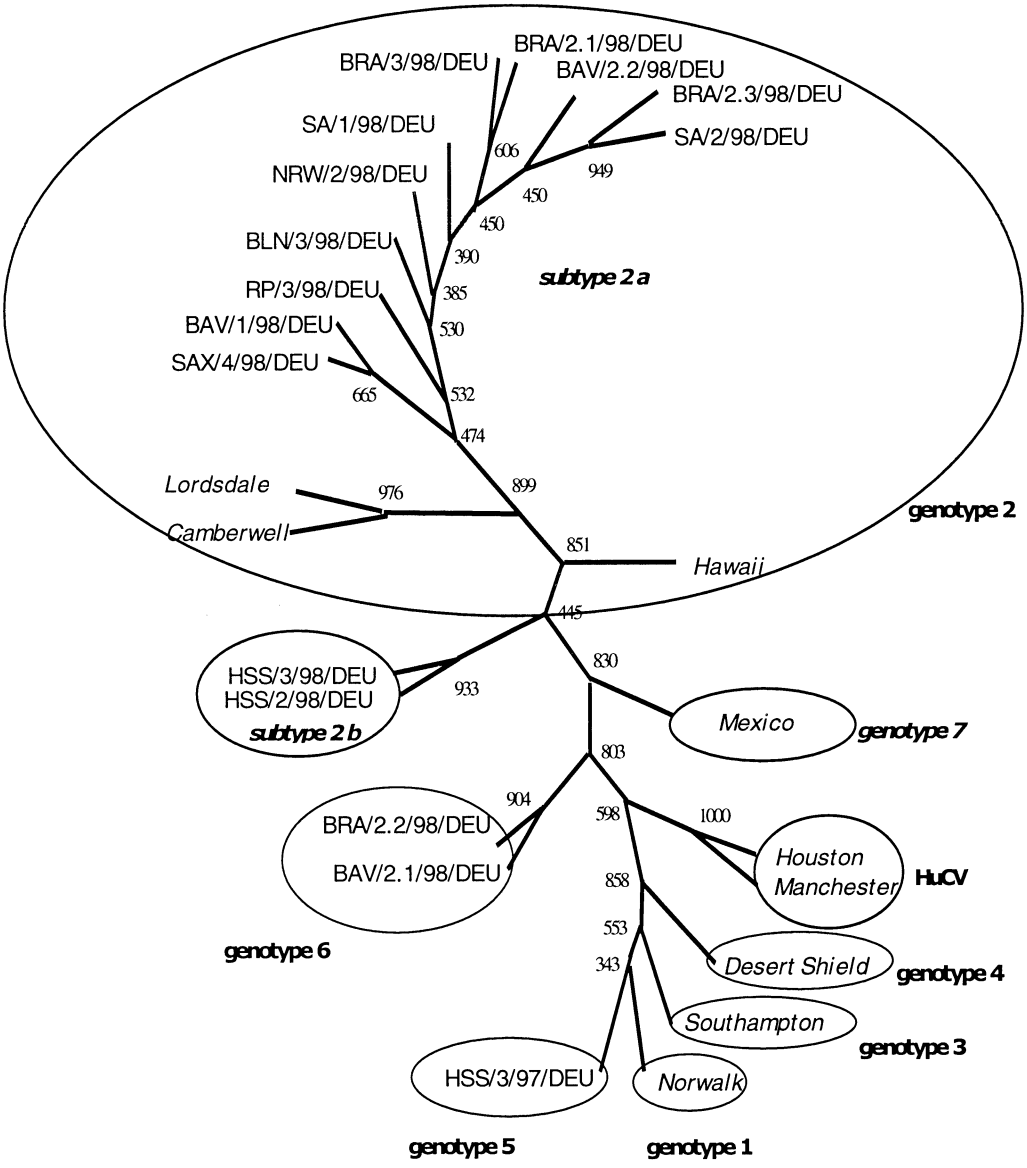

Fig. 1. Phylogenetic analysis, produced by the program of the PHYLIP package, of sequence data of ORF1 from German outbreak specimens (indicated by the ending/DEU) and GenBank database for typical members of caliciviruses. The corresponding GenBank accession numbers are: Norwalk (M87661), Lordsdale (X86557), Camberwell (U46500), Mexico (U22498), Southampton (L07418), Desert Shield (U04469), Hawaii (U07611), Houston (U95644), Manchester (X86560). Selected sequence data of SRSVs described in this article are deposited in the GenBank data libraries (Acc. nos. HSS/3/97/DEU, AF093797 [= complete sequence]; BAV/2.1/98/DEU, AF178968; HSS/2/98/DEU, AF178969; BRA/2.1/98/DEU, AF178970)

well/Aus/94 virus in the ORF1 (e.g. BRA/2.1/98/DEU; GenBank Acc. no. AF178970). The viruses were classified as genotype 2 isolates and clustered into one subtype, here termed subtype 2a (Fig. 1). Isolates from the outbreaks HSS/2/98/DEU (GenBank Acc. no. AF178969) and HSS/3/98/DEU form one branch of the tree termed subtype 2b. SRSV sequences from the three remaining outbreaks cluster in two additional genotypes; sequences of the outbreaks BAV/2.1/98/DEU (GenBank Acc. no. AF178968) and BRA/2.2/98/DEU form genotype 6 and HSS/3/97/DEU genotype 5, respectively (see Fig. 1). We have determined the complete sequence for HSS/3/97/DEU (GenBank Acc. no. AF093797, data not shown here). Overall, our sequence data of the ORF1 together with representative published SRSV sequences clustered into seven genotypes in an unrooted phylogenetic tree generated by the neighbor-joining method (PHYLIP). Genotype 2, representative of 80% of our isolates, could be divided into two subtypes. Sequence data of one isolate at a time as representatives of the genotypes 2 (subtypes 2a, 2b), 5, and 6 were published in the GenBank.

Similar results were obtained in the phylogenetic analysis using part of the ORF3 (Fig. 2). The majority of German isolates also cluster in genotype 2. Compared to the ORF1 region, an alteration was only found for the isolates of the outbreak BRA/2.2/98/DEU which in ORF3 are closely related to genotype 2 (subtype 2a) and for isolates of outbreaks in Hesse (HSS/2/98/DEU and HSS/3/98/DEU) which do not form a distinct subtype within genotype 2. In this context it is remarkable that, while the ORF1 sequence of the isolate Hawaii/USA/71 (GenBank Acc. no. U07611) clustered into genotype 2, the sequence of ORF3 is quite different from all sequences of isolates of genotype 2. The greatest homology (nt: 77.4%; aa: 75.3%) was found with the German isolate BAV/2.1/98/DEU (GenBank Acc. no. AF178971) and MX/Mexico (nt: 70%; aa: 72.5%; GenBank Acc. no. U22498). Neighbor-joining analysis of the deduced amino acid sequences of ORF1 and ORF3 essentially results in identical trees compared to trees of nucleotide sequences (not shown here). The protein alignment of part of the ORF1 (Fig. 3) indicates for all German isolates belonging to genotype 2 – including Lordsdale, Camberwell and Hawaii – a relatively highly conserved amino acid sequence. The same is also true for the isolates investigated belonging to genotype 6 from Brandenburg (BRA/2.2/98/DEU) and from Bavaria (BAV/2.1/98/DEU).

With regard to the ORF3 part, the protein alignment (Fig. 4) also shows for all SRSVs classified as genotype 2 relatively highly conserved amino acid sequences. In the ORF3 the sequence from Hawaii partly corresponds with both BAV/2.1/98/DEU and MX/Mexico. Overall, the amino acid sequences of all 7 genotypes defined here are different from each other.

Discussion

The development and design of molecular methods of detection and characterization of SRSVs increased our understanding of their molecular epidemiology. Presently, extensive sequence comparison of caliciviruses has suggested

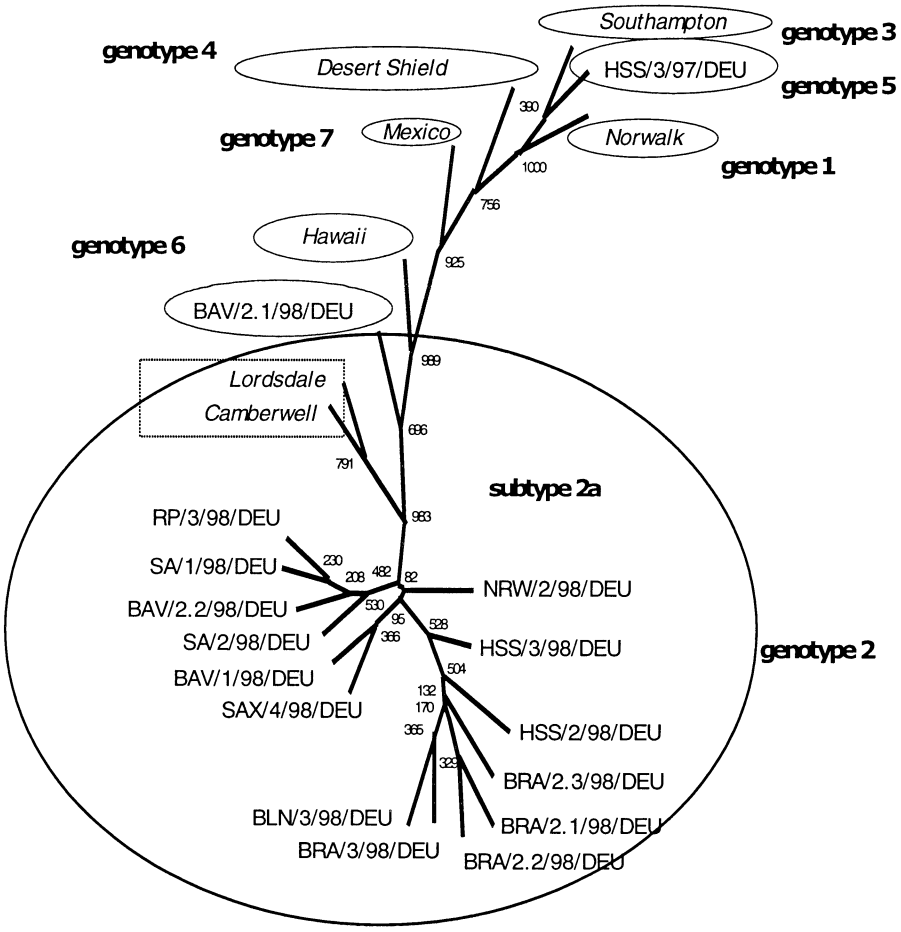

Fig. 2. Phylogenetic analysis, produced by the program of the PHYLIP package, of sequence data of ORF3 from German outbreak specimens and GenBank database for typical members of SRSVs (see also legend to Fig. 1). Selected sequence data of SRSVs described in this article are deposited in the GenBank data libraries (GenBank Acc. Nos. HSS/3/97/DEU, AF093797 [= complete sequence]; BAV/2.1/98/DEU, AF178971; BRA/2.1/98/DEU, AF178972)

that SRSVs could be subdivided phylogenetically into 2 genogroups while classical human caliciviruses (HuCV) have been assigned to genogroup 3 [9, 22].

In our study we investigated a total of 16 outbreaks of gastroenteritis in 1997 and 1998 by RT-PCR and direct sequence analysis in two different genome regions. Although several study groups have described PCR systems for the detection of SRSVs [1, 8, 18, 20], we designed in-house primer pairs for a region of the RNA-dependent RNA polymerase gene (ORF1) and the small basic protein (ORF3). While our PCR system in ORF1 detects SRSVs belonging to both genogroup I and genogroup II, the primer pairs in the ORF3 should be only specific for genogroup II sequences. This allows a first orientation about the as-

	1538							1615
Norwalk	SFSIMSRLTA	SPELAEVVAQ	DLLAPSEMDV	GDYVIRVKEG	LPSGFPCTSQ	VNSINHWIIT	LCALSEA•••	••TGLSPDVV QSM
Southampton	-----C----	-----S----	----------	----------	----------	-------L--	------V•••	••-------I ---
Desert Shield	--N--CK---	N-S--A----	---S------	-----S--D-	----------	--------L-	------V•••	••-------L --Q
HSS/3/97/DEU	-----C----	--D--A---R	----------	--F-------	----------	-------L--	------V•••	••---E---I --Q
MX/Mexico	ALE--V-FS-	E-Q--QI--E	------VV-E	--FK-TIN--	----V-----	R--TA--LL-	------V•••	••---G--II -AN
Lordsdale	ALE--VKFSP	E-H--QI--E	---S--V---	--FK-SIN--	----V-----	W---A--LL-	------V•••	••-N----II -AN
Camberwell	ALE--VKFSP	E-H--QI--E	---S--V---	--FK-SIN--	----V-----	W---A--LL-	------V•••	••-N----II -AN
Hawaii	ALE--VKFSP	E-H--Q---E	---S--V---	--FK-SIN--	----V-----	W---T--LL-	------V•••	••-D----II -AN
RP/3/98/DEU	ALE--VKFSS	E-H--Q---E	---S--VV--	--FK-SIN--	----V-----	W---A--LL-	------V•••	••-N----II -AN
SAX/4/98/DEU	ALE--VKFSS	E-H--Q---E	---S--VV--	--FK-SIN--	----V-----	W---A--LL-	------V•••	••-N----II -AN
BAV/1/98/DEU	ALE--VKFSS	E-H--Q---E	---S--VV--	--FK-SIN--	----V-----	W---A--LL-	------V•••	••-N----II -AN
BLN/3/98/DEU	ALE--VKFSS	E-H--Q---E	---S--VV--	--FK-SIN--	----V-----	W---A--LL-	------V•••	••-N----II -AN
BAV/2.2/98/DEU	DQE--VKFSS	E-H--Q---E	---S--VV--	--FK-SIN--	----V-----	W---A--LL-	------V•••	••-N----II -AN
BRA/2.1/98/DEU	ALE--VKFSS	E-H--Q---E	---S--VV--	--FK-SIN--	----V-----	W---A--LL-	------V•••	••-N----II -AN
BRA/3/98/DEU	ALE--VKFSS	E-H--Q---E	---S--VV--	--FK-SIN--	----V-----	W---A--LL-	------V•••	••-N----II -AN
NRW/2/98/DEU	ALE--VKFSS	E-H--Q---E	---S--VV--	--FK-SIN--	----V-----	W---A--LL-	------V•••	••-N----II -AN
SA/1/98/DEU	ALE--VKFSS	E-H--Q---E	---S--VV--	--FK-SIN--	----V-----	W---A--LL-	------V•••	••-N----II -AN
SA/2/98/DEU	ALE--VKFSS	E-H--Q---E	---S--VV--	--FK-SIN--	----V-----	W---A--LL-	------V•••	••-N----II -AN
BRA/2.3/98/DEU	ALE--VKFSS	E-H--Q---E	---S--VV--	--FK-SIN--	----V-----	W---A--LL-	------V•••	••-N----II -AN
HSS/2/98/DEU	ALE-LVKFSS	E-H--Q---E	---S--V---	--FK-SIT--	----V-----	W---A--LL-	------T•••	••-N----II -AN
HSS/3/98/DEU	ALE-LVKFSS	E-H--Q---E	---S--V---	--FK-SIT--	----V-----	W---A--LL-	------T•••	••-N----II -AN
BAV/2.1/98/DEU	AMEV-V-FS-	E----Q---E	-------L--	--FI-S-Q--	----V-----	W---A---L-	-S-MA-V•••	••S------- -AH
BRA/2.2/98/DEU	AMEV-V--S-	E----Q---E	-------L--	--FI-S-Q--	----V-----	W---A---L-	-S-MA-V•••	••S------- -AH
Manchester	-LA-LE-FAE	PHPIVSCAIE	A-SS-A-GY-	N-IKFVTRG-	----M-F--V	------M-YV	AA-ILQ-•••	••YE•-HN-P YTG
Houston	-L--LE-FME	-SP-VSCAIE	S-SS-AIGYL	N-IKFVT-G-	----M-F-LV	I--V--M-YF	AAGVLK-YED	HHVPYTGN-F -IE

Fig. 3. Alignment of amino acid sequences of ORF1 of German outbreak specimens and GenBank database for typical members of caliciviruses. Sequence alignments were generated using Clustal W included in the MacVector sequence analysis package. Dashes indicate identity to the Norwalk sequence; dots indicate gaps. The positions of amino acids correspond to the whole protein derived from ORF1 of Norwalk viruses (GenBank Acc. no. M87661). For further information see legend of Fig. 1 and Table 1

	22								98
Norwalk	IQVGGEAALQ	SQRYQQNLQL	QENSFK····	·····HDR	EMIGYQVEAS	NQLLAKNLAT	RYSLLRAGGL	TSADAARSVA	GAPVTRIV
Southampton	--A-A-----	------D-A-	QR-T-E····	·····--K	D-LS---Q--	-A------N-	---M-I----	S----S-A--	------LI
Desert Shield	--A-T-----	H--F--D-T-	-S---K····	·····--K	----L--G--	TA--QNS-N-	--N--TQA-M	STT----A-V	---T--V-
HSS/3/97/DEU	--A-A--G--	A-----D---	-Q-T-Q····	·····---	-------Q--	-A------KS	--T--Q----	-------A--	--------
MX/Mexico	VNA-AN-IN-	KVDFEN-KY-	RNA--N····	·····--K	--LNA-IA-T	KR-Q-DMI-I	KQPV-TLAAF	PLL·MQHVAI	N--M-KVL
Lordsdale	-NA-AG-IN-	KVEFEN-RK-	-QA--QFSST	LQQASFQ--K	--LQA-I--T	QK-QQDLMKV	KQAV-LE--F	ST-----GAI	N--M-KAL
Camberwell	-NA-AG-IN-	KVEFEN-RK-	-QA--QFSSN	LQQASFQ--K	--LQA-I--T	QK-QQGLMKV	KQAM-LE--F	STT----GAI	N--M-KAL
BRA/2.2/98/DEU	-NA-AG-IN-	KVEFEN-RK-	-QA--QFSSN	LQQASFQ--K	--LQA-I--T	-K-QQGMMKI	KQAM-LE--F	SET----GAI	N--M-KAL
HSS/2/98/DEU	-NA-AG-IN-	KVEFEN-RK-	-QA--QFSSN	LQQASFQ--K	--LQA-I--T	K--QQGMMKV	KQAM-LE--F	SET----GAI	N--M-KAL
HSS/3/98/DEU	-NA-AG-IN-	KVEFEN-RK-	-QA--QFSSN	LQQASFQ--K	--LQA-I--T	-K-QQGMMKI	KQAM-LE--F	SET----GAI	N--M-KAL
RP/3/98/DEU	-NA-AG-IN-	KVEFEN-RK-	-QA--QFSSN	LQQASFQ--K	--LQA-I--T	KS-QQGMMKV	KQAM-LE--F	SET----GAI	N--M-KAL
SAX/4/98/DEU	-NA-AG-IN-	KVEFEN-RK-	-QA--QFSSN	LQQASFQ--K	--LQA-I--T	K--QQGMMKV	KQAM-LE--F	SET----GAI	N--M-KAL
BAV/1/98/DEU	-NA-AG-IN-	KVEFEN-RK-	-QA--QFSSN	LQQASFQ--K	--LQA-I--T	K--QQGMMKV	KQAM-LE--F	SET----GAI	N--M-KAL
BLN/3/98/DEU	-NA-AG-IN-	KVEFEN-RK-	-QA--QFSSN	LQQASFQ--K	--LQA-I--T	-K-QQGMMKI	KQAM-LE--F	SET----GAI	N--M-KAL
BAV/2.2/98/DEU	-NA-AG-IN-	KVEFEN-RK-	-QA--QFSSN	LQQASFQ--K	--LQA-I--T	KN-QQGMMKV	KQAR-LE--F	SET----GAI	N--M-KAL
BRA/2.1/98/DEU	-NA-AG-IN-	KVEFEN-RK-	-QA--QFSSN	LQQASFQ--K	--LQA-I--T	-K-QQGMMKI	KQAM-LE--F	SET----GAI	N--M-KAL
BRA/3/98/DEU	-NA-AG-IN-	KVEFEN-RK-	-QA--QFSSN	LQQASFQ--K	--LQA-I--T	-K-QQGMMKI	KQAM-LE--F	SET----GAI	N--M-KAL
NRW/2/98/DEU	-NA-AG-IN-	KVEFEN-RK-	-QA--QFSSN	LQQASFQ--K	--LQA-I--T	KK-QQGMMKV	KQAM-LE--F	SET----GAI	N--M-KAL
SA/1/98/DEU	-NA-AG-IN-	KVEFEN-RK-	-QA--QFSSN	LQQASFQ--K	--LQA-I--T	KN-QQGMMKV	KQAM-LE--F	SET----GAI	N--L-KAL
SA/2/98/DEU	-NA-AG-IN-	KVEFEN-RK-	-QA--QFSSN	LQQASFQ--K	--LQA-I--T	KN-QQGMMKV	KQAM-LE--F	SET----GAI	N--M-KAL
BRA/2.3/98/DEU	-NA-AG-IN-	KVEFEN-RK-	-QA--QFSSN	LQQASFQ--K	--LQA-I--T	-K-QQGMMKI	KQAM-LE--F	SET----GAI	N--M-KAL
BAV/2.1/98/DEU	-NA-AN-VN-	KVE-DF-K--	-MA--------	·····--K	--LQS--L-T	K--QQEMMNI	-QGV-T---F	SP-----GAV	N--M-KAL
Hawaii	VNA-AN-IN-	KVDFEN-K--	-QA--N····	·····--K	--LQA-IQ-T	K--Q-DMI-L	-QGV-T---F	SPT----GAV	N--M-QVL

Fig. 4. Alignment of amino acid sequences of ORF3 of German outbreak specimens and GenBank database for typical members of caliciviruses. The positions of amino acids correspond to the whole protein derived from ORF3 of Norwalk viruses. For further information see legend of Figs. 1, 3 and Table 1

signment of isolates to a genogroup. The amplificates for ORF1 and ORF3 which are about 300 bp long should be representative in order to allow differentiation between outbreak strains.

Our own findings that most outbreaks studied were associated with genogroup II viruses correlate with investigations in the US, UK, The Netherlands and Australia which also found a predominance of genogroup II SRSV-associated outbreaks [1, 7, 10, 17, 19–21, 23].

All specimens collected in a single outbreak showed 100% nucleotide identity in the ORF1 and ORF3 region of SRSVs. It has to be mentioned that the sequences were exclusively determined by direct sequencing and not after molecular cloning. However, the excellent readability of the sequences argues for a small quasispecies population. Isolates from unrelated outbreaks showed a varying degree of homology. However, most isolates of different outbreaks generally showed a $> 95\%$ homology on the nucleotide level both in the ORF1 and ORF3, forming a cluster within genogroup II (SRSVs). These findings correlated with investigations of gastroenteritis outbreaks in the Netherlands, Australia and the United Kingdom between 1995 and 1997 [17, 23] which indicated an epidemic spread of closely related SRSVs.

Recently, Noel et al. [19] reported the global distribution of a distinct common strain of "Norwalk-like virus". In this context it is remarkable that the majority of the SRSV sequences investigated in the present study are almost identical to sequences of SRSV outbreaks reported in the United States, Australia, The Netherlands, the United Kingdom, Japan or Korea. The dominance of SRSV strains belonging to genogroup II in current viral gastroenteritis outbreaks worldwide is also true for Germany. However, SRSVs quite distinct from genogroup I as well as from genogroup II viruses are circulating and could be causing SRSV-associated outbreaks of gastroenteritis in Germany. We were able to show this by our sequence data in the ORF1 and ORF3 region in specimens from three different outbreaks. Divergences of about 25% to 50% from genogroups I and II reveal the extent of variability of the SRSVs.

Based on the degree of variability, we classified our isolates and published SRSVs of genogroup I and II into different genotypes and subtypes. Isolates of the same subtype showed nucleotide sequence similarities in the ORF1 and ORF3 of about $\geq 88\%$, whereas those between different genotypes was $\leq 77\%$. Based on the reactivity of primers for the RT-PCR and oligonucleotide probe sets for Southern hybridization as well as an 81 bp sequence from the RNA polymerase gene, Ando et al. [1] classified SRSVs of the genogroups 1 and 2 into two phylogenetic groups each. Wright et al. [23] divided SRSVs of genogroup 2 into two subgroups; one of them (2A) containing Mexico-like viruses, the other (2B) Lordsdale/Camberwell-like viruses. The majority of viruses detected in Australia belonged to the genogroup 2B.

It is interesting that the genetic relatedness of SRSV isolates is not always equivalent when analyzing different genome regions. The fact that our isolates BRA/2.2/98/DEU and BAV/2.1/98/DEU are highly identical in the ORF1 but quite different in the ORF3 stresses the necessity to perform the molecular analysis

in at least two different genome regions in order to be able to differentiate properly between outbreak strains.

The discordance of genotype affiliation of some SRSVs shown in ORF1 and ORF3 may suggest intertypic recombination events in vivo. Such recombination events are known for single-stranded RNAs of positive polarity [3, 15].

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