Arch Virol (2004) 149: 155–172
DOI 10.1007/s00705-003-0199-1

Archives of Virology
Printed in Austria

Molecular characterization of a human rotavirus reveals porcine characteristics in most of the genes including VP6 and NSP4

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Received March 27, 2003; accepted July 1, 2003
Published online September 22, 2003

Summary. Long electropherotype with Subgroup I specificity is a common feature of animal rotaviruses. In an epidemic of infantile gastroenteritis in Manipur, India, long but SG I strains predominated in the outbreak in the year 1987–88. One such strain isolated from that region, following the outbreak had G9P [19] specificity. As this is a rare combination, the gene sequences encoding VP4, VP6, VP7, NSP1, NSP2, NSP3, NSP4 and NSP5 of this strain were analyzed. All these genes except VP7 were closely related to porcine rotaviruses (95–99% identity at amino acid level) and clustered with the porcine strains in phylogenetic analysis. In addition, it had subgroup I nature and belonged to NSP4 genotype B which is characteristic of animal rotaviruses. This is the first report of a rotavirus with VP6 and NSP4, two crucial proteins thought to be involved in host range restriction and pathogenicity, were of porcine origin and caused diarrhoea in a human host. Among the genes of this strain sequenced so far, only VP7 had highest identity to human strains at amino acid level. This study suggests reassortment may be occurring between human and other animal strains and some of the reassortant viruses may be virulent to humans.

Introduction

Rotaviruses are the major cause of severe gastroenteritis in young children and animals [19]. In humans rotavirus diarrhoea results in significant morbidity and mortality, especially in developing countries [19]. Rotavirus genome consists of 11 segments of double stranded RNA, each encoding a viral protein, enclosed in a triple layered viral capsid [10]. There are seven groups (A–G) and two major subgroups (I and II) of Group A rotaviruses which are defined by antigenicity of inner capsid protein VP6 [10]. Among them only Group A, B and C have been detected in human [19]. The segmented nature of the rotavirus genome provides a unique mechanism for the generation of genetic diversity by the process of genetic reassortment that occurs during mixed infections *in vivo* as well as *in vitro* [32]. Reassortment among co-circulating strains contributes significantly to the overall genomic diversity of rotaviruses [17].

Two distinct RNA profiles, a long electropherotype and a short electropherotype are associated with differences in the mobility of the gene coding for NSP5 which corresponds to segment 11 for long electropherotype and segment 10 for short electropherotype strains [10]. Ghosh and Naik [12] reported an outbreak of rotaviral diarrhoea in Manipur, India, in which most of the strains (>60%) had long electropherotype with subgroup I specificity. Among human rotaviruses subgroup I specificity is associated with short electropherotype and subgroup II with long electropherotype. On the other hand, long electropherotype with subgroup I specificity is usually found in animal rotaviruses [19] and hence these strains were thought to have originated from an animal reservoir [12]. In a subsequent epidemiological study (1989–1992) such strains were still circulating within the population at a low frequency (2.1%) [22]. Clustering of rotavirus strains according to species of origin have been reported for the rotaviral genes encoding VP4, VP6, VP7, NSP1, NSP3 and NSP4 [6, 13, 14, 21, 33, 38, 43].

In the present study, we sequenced the genes coding for VP4, VP6, VP7, NSP1, NSP2, NSP3, NSP4 and NSP5 proteins of one such isolate RMC321 and compared the deduced amino acid sequences with other known sequences deposited in the GenBank database. Here we report detection of a human rotavirus strain from an infant with diarrhoea where majority of the gene sequences showed significant identity to porcine rotaviruses.

Materials and methods

Viruses

The isolate RMC321 was collected from Manipur, India, as part of a molecular epidemiological study (1989–1992) following the outbreak of infantile gastroenteritis with human rotaviruses having long electropherotypes but SG I characteristics in 1987–1988 [12]. Out of the 305 stool samples collected from the Regional Medical College, Imphal, Manipur, 102 samples were positive for rotaviruses, of which 5 samples were long electropherotype with SG I specificity [22]. The stool samples were tested negative for all bacterial and parasitic pathogens; therefore rotavirus was the sole pathogen for diarrhoea.

Table 1. List of primers used for amplification and sequencing of various genes of the rotavirus strains. The positions of the consensus sequences are represented with reference to a known sequence

Primer	Sequence	Polarity	Nucleotide position	Size	Gene	Reference strain
VP4IF	TAATATATCTAGATATGAAGTG	+	1017–1038	22mer	VP4	Mc323
VP4IR	GCTCTACAGTAGTCGAGTCA	−	196–177	20mer		Mc323
VF5F	GAAAAGTCTTGTGGAAGCCATG	+	14–35	22mer	NSP1	69M
VF5R	GGTCACATTTTATGCTGCCTA	−	1567–1547	21mer		69M
VFI1	ATGATTCACACTTTTCCATTAATG	+	702–725	24mer		IGV-80-3
VFI2	CATCTGGTAGAAACACTATAAAATTC	−	891–866	26mer		IGV-80-3
VFI3	CTGTATGGAATGATTTTAGAATTAAG	+	1023–1048	26mer		IGV-80-3
VFI4	AGTTAATATTTGTGGTACTTTACC	−	1327–1304	24mer		IGV-80-3
VF3F	GGCTTTTAAAGCGTCTCAGTC	+	1–21	21mer	NSP2	KU
VF3R	GGTCACATAAGCGCTTTCTATTC	−	1058–1036	23mer		KU
VFI2F	TAGCAATAAAATGTATGTTGACAGC	+	119–143	25mer		KU
VFI2R	GCAACTGATGAATATTTAACAACTC	−	749–725	25mer		KU
VF2F	ATGCTCAAGATGGAGTCTACT	+	1–21	21mer	NSP3	Wa
VF2R	GGTCACATAACGCCCCTATAG	−	1050–1030	21mer		Wa
VFI3F	AATAGAAATTGGATGACTGATTCT	+	250–273	24mer		Wa
VFI3R	AGATACCACTCAACTGATGACAC	−	746–724	23mer		Wa
VF4F	GGCTTTTAAAAGTTCTGTTCCG	+	1–22	22mer	NSP4	Wa
VF4R	GGTCACACTAAGACCATTCCT	−	750–730	21mer		Wa
VF1F	GGCTTTTAAAGCGCTACAGTG	+	1–21	21mer	NSP5	KU
VF1R	GGTCACAAAACGGGAGTGGG	−	664–645	20mer		KU

Viral RNA extraction, cDNA synthesis and PCR amplification

Total viral RNA was extracted from the stool sample using a commercially available RNA extraction kit (BIO 101, La Jolla, CA, U.S.A.) following manufacturer's instructions with some modifications as mentioned earlier [36]. Reverse Transcription (RT) and Polymerase Chain Reaction (PCR) were carried out according to manufacturer's protocols using Superscript II reverse transcriptase (Invitrogen Corporation, Carlsbad, CA, U.S.A.) and Platinum Taq DNA Polymerase (Invitrogen Corporation, Carlsbad, CA, U.S.A.). The primers for NSPs were designed from known sequences from the GenBank database as consensus oligomers (Table 1). For VP6 gene, the primers used by Shen et al. [37] were used without the linkers. Amplification of VP4 was carried out as described earlier [42] and the internal primers used for sequencing VP4 given in Table 1. Full length VP7 was amplified as described by Taniguchi et al. [39].

Cloning and sequencing

The full-length genes were cloned into pCR2.1 Vector using the TOPO-TA cloning kit (Invitrogen Corporation, Carlsbad, CA, U.S.A.). The plasmid with the insert was isolated using QIAprep Spin Miniprep plasmid isolation kit (QIAGEN Inc., Valencia, CA, U.S.A.). Sequencing was carried out using ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kits (PE Applied Bio Systems, Foster City, California, U.S.A.) with the ABI Prism 310 Genetic Analyzer (PE Applied Bio Systems, Foster City, California, U.S.A.). Three clones were sequenced for each gene to avoid ambiguities.

Sequence analysis

BLAST was used for homology search of the Nucleotide and Amino acid sequences [1]. Multiple alignments were carried out using the programme Clustal X version 1.8 with default parameters. To calculate the amino acid identity in percentage, pair wise alignment was carried out using William Pearson's lalign program with the global alignment option (http://www.ch.embnet.org/software/LALIGN_form.html). Phylogenetic tree was constructed using the Neighbor Joining method [34] (500 bootstrap trials, branch lengths evaluated using Fitch-Margoliash method) in the DAMBE software (Version 4.0.75) and tree was drawn with Tree View Version 1.6.6 (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html). All the trees were rooted with the respective proteins of avian rotavirus PO13. The GenBank accession numbers of the sequences reported in this paper are AF523677, AF501578, AF531913, AY033396, AF506292, AF506293, AF541920 and AF541921.

Results

Sequence analysis of VP4

The deduced VP4 protein had maximum identity with two P [19] human rotavirus strains Mc345 and Mc323 (98.7% and 97.5% identity respectively). It also had 95.6% identity with the porcine strain 4F, the first P [19] rotavirus isolate. With other P types, it had only 58.2–84.3% identity (Table 2).

Table 2. Percent amino acid identity of the deduced amino acid sequence of the different structural and nonstructural proteins of RMC321 with other human and animal rotavirus strains. The highest identity is given in bold

Strain	Host species	VP4	VP6	VP7	NSP1	NSP2	NSP3	NSP4	NSP5
4F	Porcine	95.6	**98.7**	87.1	**94.9**				
4S	Porcine	70.9	98.5	87.4	**94.9**				
69M	Human	75.4		82.2	68.5		82.7		84.3
A34	Porcine							**98.3**	
A131	Porcine		**98.7**	86.2				95.4	
A253	Porcine		**98.7**					97.1	
A411	Porcine		94.5	86.2				95.4	
AU1	Human			86.2	56.4			84.0	90.4
BAP2	Lapine				35.2			86.9	
CC86	Porcine								97.0
CN86	Porcine		**98.7**						
CRW8	Porcine		97.0						
DS1	Human				67.7	88.3			83.3
E210	Human		94.2						
EW	Murine	72.3	87.7	84.4	36.6			63.4	
FI14	Equine		90.9		34.1				
FRV64	Feline	76.5			34.8			81.1	
GO	Porcine						**95.2**		
Gottfried	Porcine	83.5	93.2	80.1	75.7				

(*continued*)

Table 2 (*continued*)

Strain	Host species	VP4	VP6	VP7	NSP1	NSP2	NSP3	NSP4	NSP5
H1	Equine	72.0	98.2	83.1	73.5			96.6	
H2	Equine	74.6	89.5					84.6	
I321	Human	58.2	90.2	80.7	77.8	88.2	93.0	84.0	
IS2	Human		90.2			85.5	80.5		
K8	Human	66.8		82.2	79.0				89.4
K9	Canine	76.3		86.8	34.9				
KU	Human				77.0	94.3	90.4	92.6	95.4
L26	Human	79.2		82.2	68.1			82.9	
M37	Human	84.3			80.9			92.0	
MC323	Human	97.5		**97.5**					95.9
MC345	Human	**98.7**		95.1					**98.5**
MP409	Human	74.9		82.5	55.0		85.0	80.6	
NCDV	Bovine		90.2	83.4		87.7	88.5		
OSU	Porcine	73.3	98.5	82.5	81.1	**95.9**	94.6	97.7	95.9
PO13	Avian	59.3	74.3	59.5	17.1	56.5	44.5	31.8	49.5
RF	Bovine	75.1	90.2	82.8		87.1	89.1		89.9
RRV	Simian				35.7		84.0	82.9	92.9
RV3	Human	84.1	93.2					92.6	
S2	Human		89.7	77.9			82.4	83.4	
SA11	Simian	76.4	90.4	87.7	33.9	89.0	78.4	83.4	90.9
ST3	Human	83.2		80.4	80.5		93.3	91.4	
UK	Human		90.7	85.0	56.6	90.5	87.9	85.7	89.9
V51	Human								83.0
V61	Human								82.5
WA	Human	80.4	93.2	81.6	78.4	95.3	92.3	95.4	95.4
X57943			92.7						
YM	Porcine	73.1	98.5	85.0	73.9			94.9	**98.5**

Sequence analysis of VP6

The deduced amino acid sequence of VP6 gene had 98.7% identity to three SG I porcine rotaviruses 4F, A131, A253 and a SG II porcine rotavirus CN86. It had 97.0–98.5% identity to other porcine strains 4S, CRW8, OSU, YM and the equine strain H1 and 90.9–94.2% identity to the human strains Wa, E210, RV3, X57943, the equine strain FI-14, and the porcine strain Gottfried. It was less related (87.1–90.7% identity) to other SG II strains like S2, IS2, I321 (human), H2 (equine), EW (murine), SA11 (simian), NCDV, UK and RF (bovine) (Table 2, Fig. 1). With the avian rotavirus PO13, it had only 74.3% identity.

Sequence analysis of VP7

The deduced protein sequence of RMC321 VP7 showed 98.5% identity to Thirteen G9 isolates from India and USA and 91.4–98.2% identity with other G9 isolates. It was less related to some of the Indian G9 isolates like INL1 and 116E

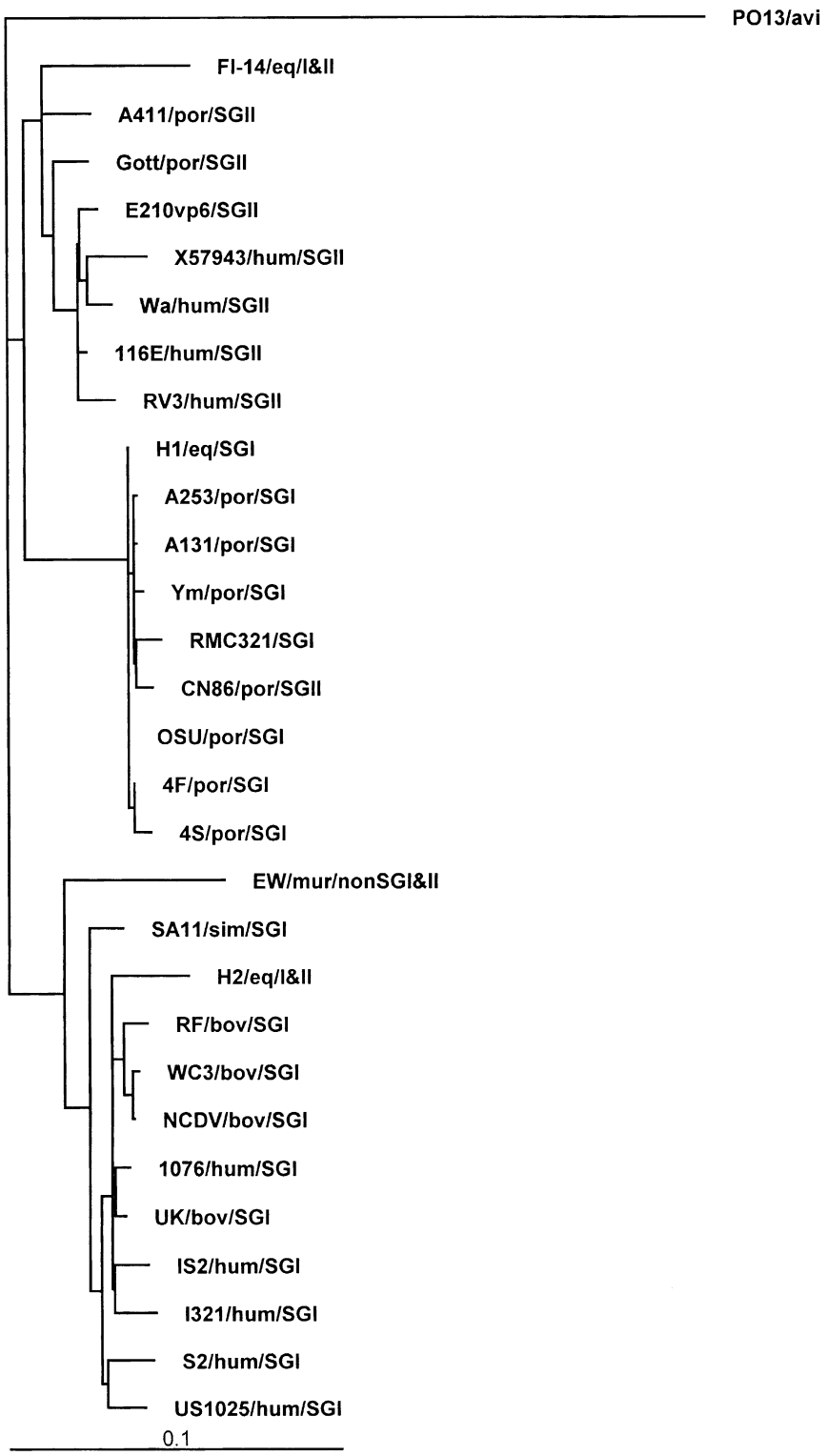

Fig. 1. Phylogenetic tree of the VP6 protein of RMC321 indicating its genetic relationship with some of the known rotavirus strains belonging to SG I, SG II, SG I&II and SG nonI&II. Noted that RMC321 clusters with porcine strains. The porcine strain Gottfried is indicated as Gott

(94.8 and 91.4% identity respectively). With other G types, it showed only 59.5–89% identity (Table 2).

Sequence analysis of NSP1 gene

The deduced protein of NSP1 gene was 481 amino acids long, 5 amino acids less than other group A rotaviruses. It had maximum identity to the NSP1 of two porcine rotavirus strains 4F and 4S at nucleotide (95.1%) and amino acid (94.9%) sequence level (Fig. 2). However, the NSP1 gene sequence of RMC 321 was shorter by 11 nucleotides at the 5′ region as the 5′ end primer was designed from nucleotide positions 14–35 of 69M NSP1 (Table 1). The NSP1 gene of RMC321 had a single extra cytosine at position 58 corresponding to an insertion between positions 69 and 70 in 4S NSP1 gene. Though the initiation codon equivalent to that in 4S was present (nucleotides 20 to 22), the reading frame terminated immediately after the insertion. The ORF initiation in RMC321 NSP1 occurs between positions 36 and 38 (corresponding to positions 62 to 64 in 4S in the −1 frame), codes for 8 amino acids and then continues into the usual reading frame after the insertion. At amino acid level, the protein had 81.1% identity to the porcine strain OSU and 73.9–75.7% identity to other porcine strains YM and Gottfried. It had 67.7–78.4% amino acid identity with the human strains DS1, 69M and Wa that clustered with the porcine strains (Table 2, Fig. 2). The Zinc finger motif was conserved except for the change in histidine to tyrosine at position 43 as found in strains like H1, L26, A44, UK and MP409 (data not shown).

Sequence analysis of NSP2 gene

RMC321 NSP2 was 95.9% identical to porcine strain OSU, differing in 13 amino acids. It clustered together in phylogenetic analysis with the human strains KU, Wa and the porcine strain OSU. Within the cluster the strains shared 93.7–96.2% identity. However RMC321 had lesser similarity to other human strains IS2, DS1 and I321 (85.5–89.7% identity) and the bovine strains RF, NCDV and UK (87.1–91.5% identity) (Table 2, Fig. 3). The cysteine residues at position 6, 8, 29, 85 and 285 were all conserved and the putative RNA binding domain (amino acid 205 to 241) was intact.

Sequence analysis of NSP3 gene

RMC 321 NSP3 had 95.2% identity to the porcine strain GO and 94.6% amino acid identity to the porcine strain OSU. It had 90.4–93.3% identity to the human strains KU, Wa and ST3. It was less related to other human rotaviruses IS2, 69M, S2 and MP409 (79.2–85% identity) as well as the bovine strains NCDV, UK and RF (86.3–89.1% identity) (Table 2, Fig. 4). The cysteine residues at 123, 139, 227 and 306 were all conserved. However, the change to isoleucine from methionine at 117 in RMC321 falls within the longest region that is completely conserved in NSP3 of all group A rotaviruses [33].

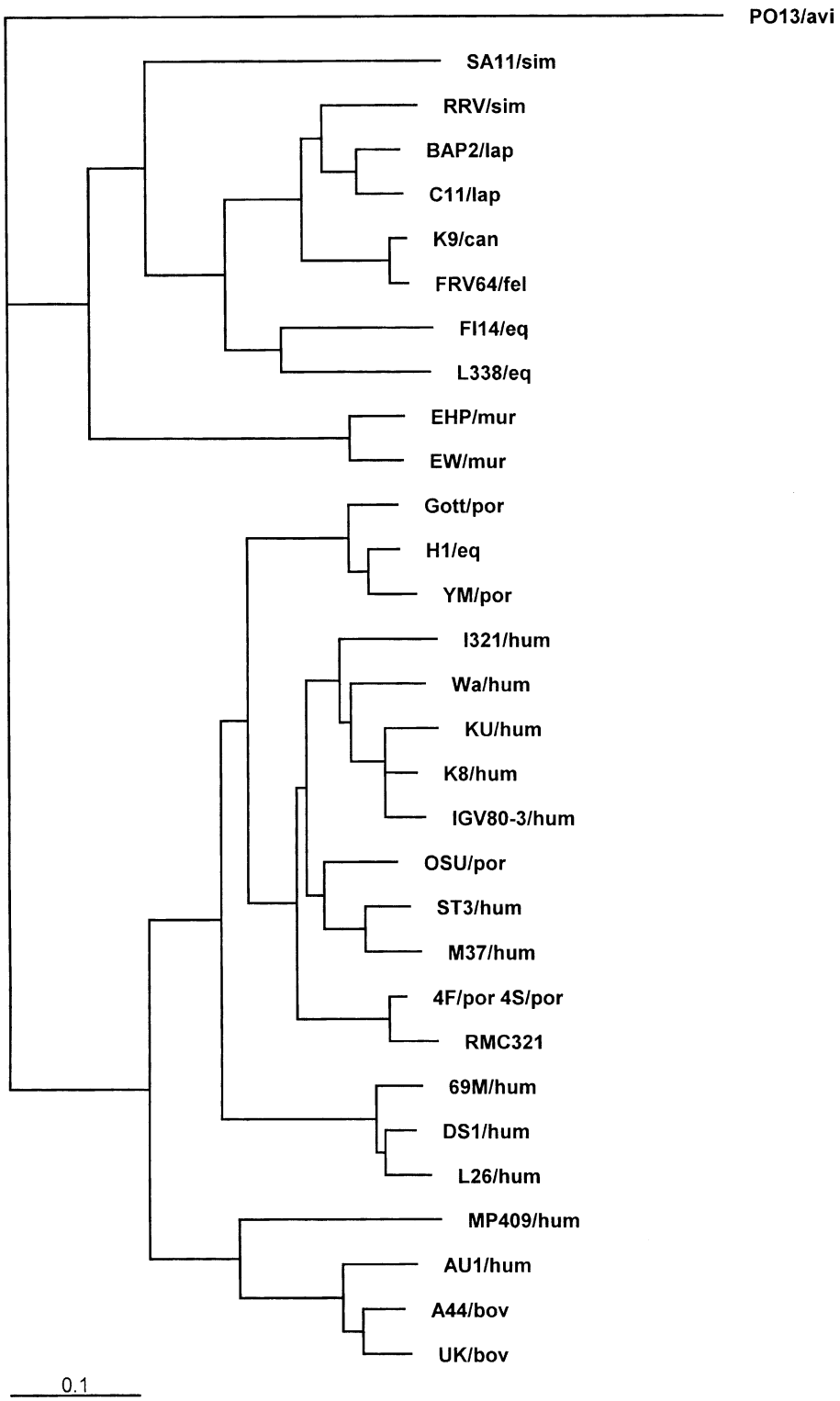

Fig. 2. Phylogenetic tree of the NSP1 protein of RMC321 indicating its genetic relationship with some of the known rotavirus strains. Noted that distinct branching of RMC321 with the porcine strains 4F and 4S. The porcine strain Gottfried is indicated as Gott

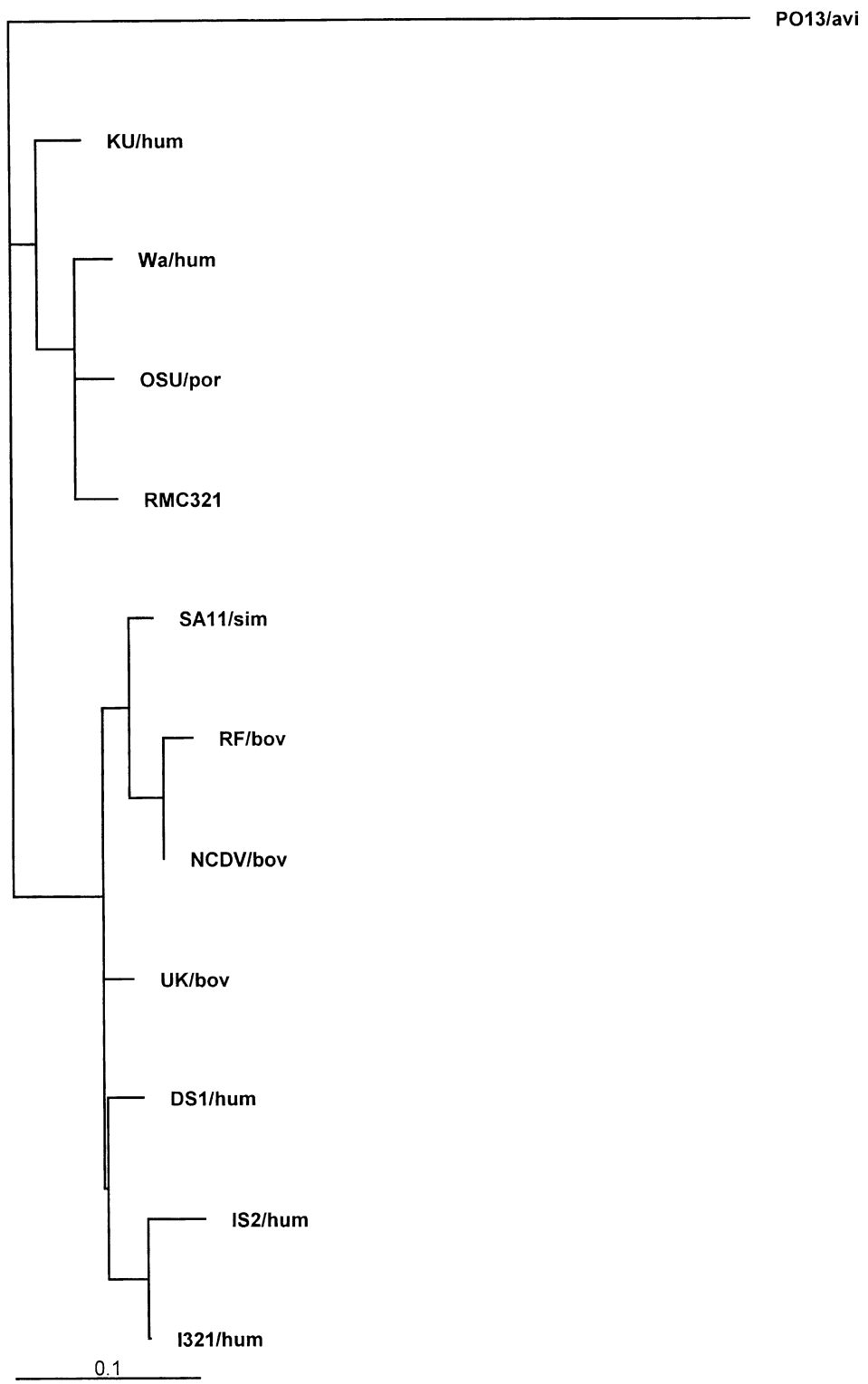

Fig. 3. Phylogenetic tree of the NSP2 protein of RMC321 indicating its genetic relationship with some of the known rotavirus strains

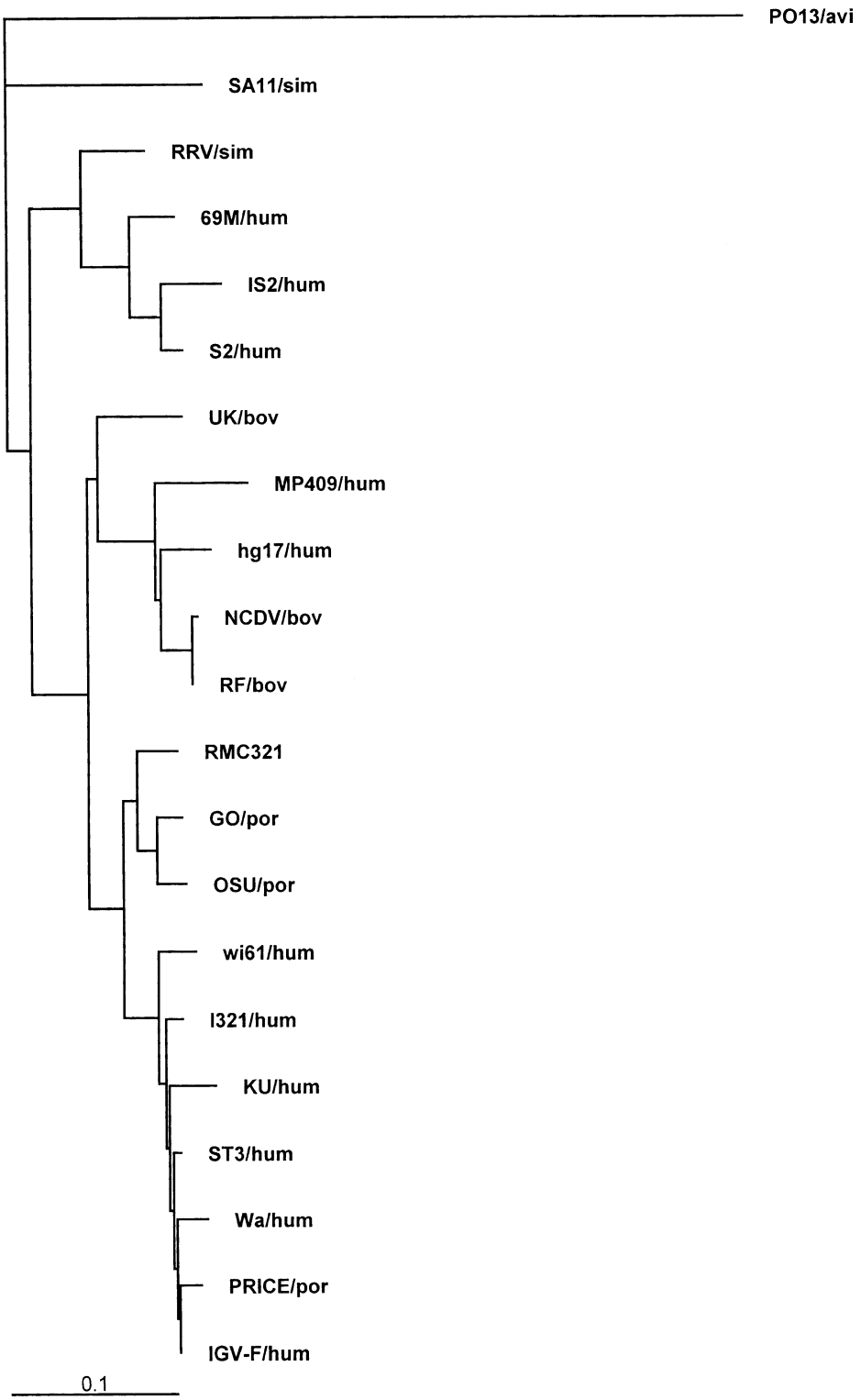

Fig. 4. Phylogenetic tree of the NSP3 protein of RMC321 indicating its genetic relationship with some of the known rotavirus strains. Noted that separate branching of RMC321 with the porcine isolates GO and OSU

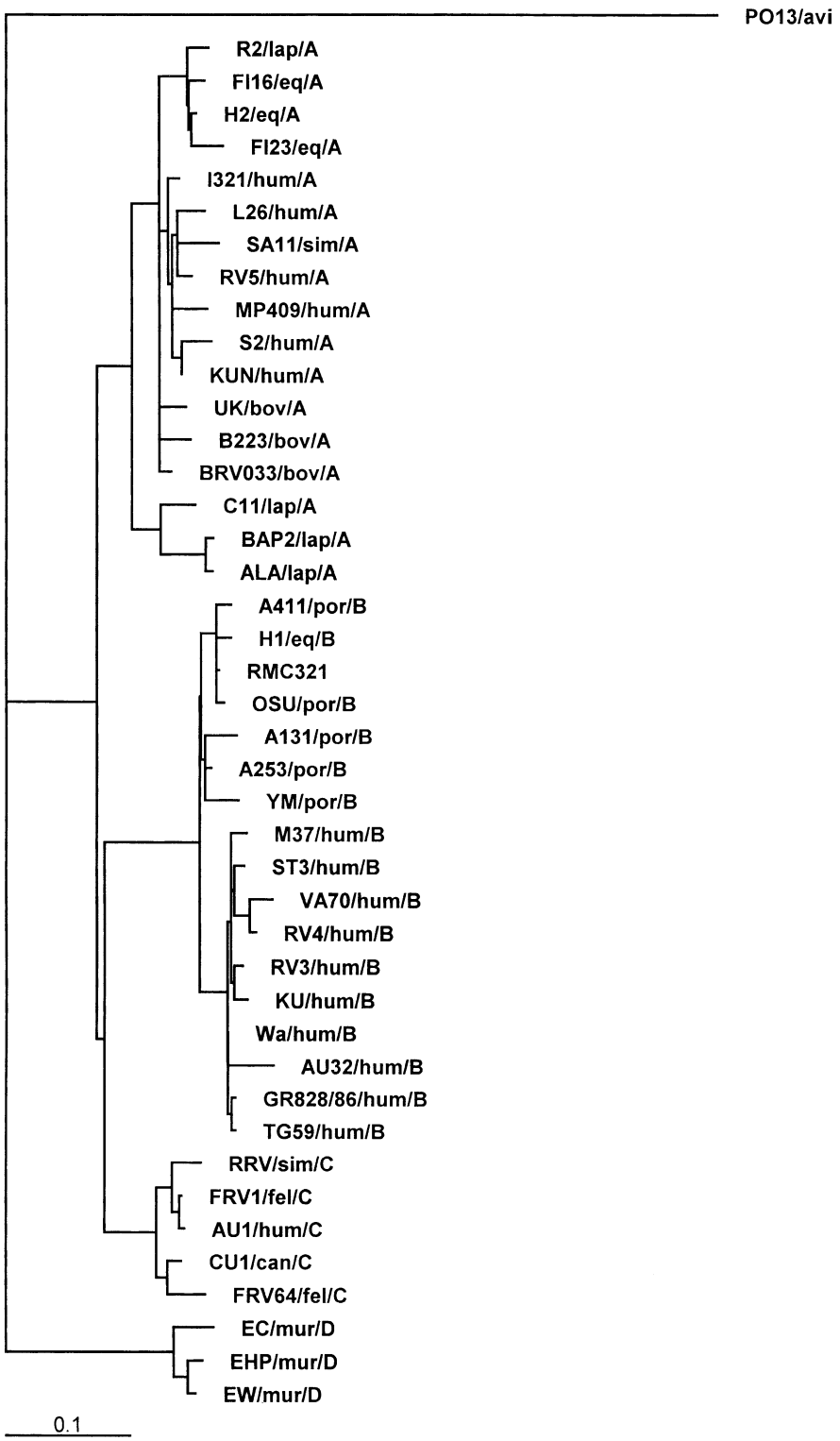

Fig. 5. Phylogenetic tree of the NSP4 protein of RMC321 indicating its genetic relationship with some of the known rotavirus strains belonging to the NSP4 genotypes A, B, C and D as indicated. RMC321 clusters with the porcine outgroup within genotype B

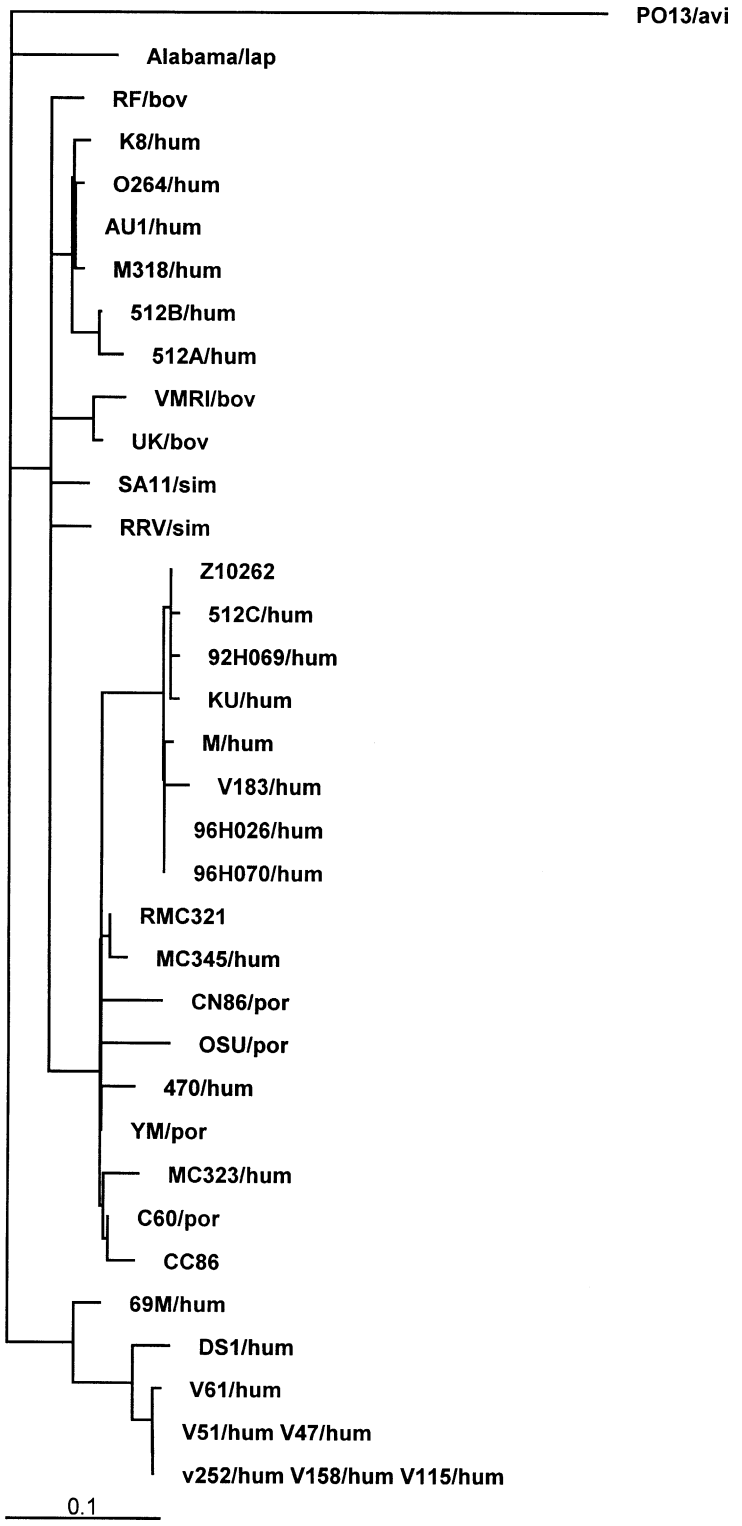

Fig. 6. Phylogenetic tree of the NSP5 protein of RMC321 indicating its genetic relationship with some of the known rotavirus strains

Sequence analysis of NSP4 gene

The NSP4 protein was closely related to porcine rotaviruses and belonged to the genotype B [6, 20] (Fig. 5). It had 98.3% amino acid identity to the NSP4 protein of A34, a G9 porcine rotavirus [6]. It also had 95.4–97.7% identity to other porcine strains A131, A253, A411, YM and OSU and 91.4–95.4% identity to the other human strains in genotype B (Table 2). In contrast RMC321 had only 83.4–85.1% identity to genotype A strains. RMC321 had potential N-linked glycosylation sites located at amino acids 8 and 18 as in other group A rotaviruses. The two cysteine residues were also conserved. However, asparagine residue at position 161 instead of serine was uncharacteristic of genotype B. Asparagine residue at this position is found in the murine strains EW and EHP (genotype D), all lapine rotaviruses, bovine rotavirus BRV033 and the human strain RV5. The variable domain [23] of RMC 321 NSP4 also had a porcine rotavirus-like consensus (AARPVDA). In the putative VP4 binding domain (amino acid 112 to 148), RMC321 NSP4 was not identical to any strain. This is not unusual as within porcine strains there is no absolute conservation in this region. Except for amino acid proline at 138 instead of serine, it was identical to the domain in OSU NSP4. This change from serine to proline was found in other porcine strains A253, A131, A34 and YM. In the enterotoxin domain (amino acid 112 to 135) [44] RMC321 was identical to porcine strain YM.

Sequence analysis NSP5

NSP5 protein of RMC321 was 98.5% identical to the human strain Mc345 and the porcine strain YM. It had 94.4–96.4% identity to other human strains like KU, 96H069, V183 and Z10262 which belonged to the same phylogenetic cluster (Table 2, Fig. 6). The strain had lesser identity (82.5–90.9%) to the human and bovine strains that clustered with AU1 (K8, 512A, RF and UK) and also to the human strains that clustered with DS1 (69M, V51, V61 and V252) (Fig. 6). The potential N-linked glycosylation sites at position 20 and 117 were intact as in other rotaviruses.

Discussion

G9 rotavirus has been associated usually with infections in humans [15] and rarely in animals [11, 31, 35]. P [19] is a rare genotype, first detected from a porcine rotavirus 4F [4]. Subsequently Okada et al. [27] reported two human P [19] strains from Thailand which shared high genetic similarity to porcine rotaviruses as per hybridization analysis [40]. The full length sequence of RMC321 VP4 was almost identical (98.7% identity) to Mc345, one of the human P [19] isolates. It is also to be noted that the human P [19] isolates also were G9 serotype. Among human rotaviruses, long and short electropherotype are linked to subgroup II and subgroup I respectively and the unusual combination of long electropherotype and subgroup I are usually of animal origin [19]. Sporadic cases of such strains infecting humans were reported and most of them were of animal origin

[24–27, 41]. However, the involvement of such strains in an outbreak of infantile gastroenteritis in Manipur, India [12], was an exceptional one.

NSP1 is the protein that exhibits substantial sequence divergence among and between the strains [16]. Previous studies on NSP1 sequences indicated it to be a host range restriction factor as it showed a clustering according to the species of origin [3, 8, 21, 43]. However comparative sequence analysis of symptomatic and asymptomatic strains revealed that although NSP1 appears to be involved in host range selection between different species, the degree of sequence variation suggests that this protein may not be solely responsible for apparent age dependent host range selection within a species [28]. NSP3 was also suggested to play a role in host range restriction of rotaviruses [33]. However, later works reported that NSP1 and NSP3 are not major determinants of host specificity between human and bovine rotaviruses [18]. As the presence of a bovine NSP1 in a porcine genetic background [2] or in the presence of porcine NSP4 and VP4 did not affect the replication in pigs, El-Attar et al. [9] suggested that NSP1 is not a determinant of host species-specific replication and disease between bovine and porcine rotaviruses. Our study extended above hypothesis to human and porcine rotaviruses.

VP6 is the major structural component of rotavirus capsid and it plays a crucial role in virion structure because of its interactions with both the outer capsid proteins VP4 and VP7 and the core protein VP2 [10]. For non-human strains VP6 shows a clustering according to species of origin [38]. The highest identity of RMC321 VP6 to a human rotavirus was 94.2 and it was with a SG II strain E210. On the other hand it had more than 98% identity to some porcine rotaviruses. In the extensive phylogenetic analysis of VP6 sequence by Tang et al. [38], three major clusters were recognized, porcine SG I cluster, SG II cluster and non-porcine SG I cluster (Fig. 1). This is the first report of a human rotavirus VP6 sequence clustering with porcine SG I rotaviruses. NSP4 sequence forms four distinct genotypes and within genotype A and B, rotavirus strains cluster according to their species of origin, suggesting a constant pattern of evolution within the species [6]. NSP4 has a role in host specific infectivity and disease [9]. The role of NSP4 in virulence and the understanding of diversity within NSP4 genotypes among species may be important as NSP4 may play a role in immunity and protection [6]. RMC 321 NSP4 clustered with the porcine rotavirus NSP4s within the genotype B, in spite of being a virulent human isolate. Among human rotaviruses, NSP4 genotype A is associated with SG I nature and NSP4 genotype B is associated with SG II nature [7, 20]. RMC321 had SG I specificity with NSP4 genotype B, typical of animal rotaviruses.

Although NSP2 of RMC321 was closely related to the NSP2 of porcine rotavirus strain OSU, (95.9% identity), it was more or less equally related to the human rotavirus strain Wa also (95.3% identity). Between them, OSU and Wa shared 96.2% identity (data not shown). The close genetic relationship between Wa and OSU NSP2 has already been reported [30]. The number of NSP2 sequences deposited in the GenBank database is less than that of other NSPs and OSU is the only porcine strain that is characterized for NSP2 so far. Therefore, the NSP2

of RMC321 could not be compared with any other porcine strain. Though NSP5 of RMC321 had 98.5% identity to the human strain Mc345, it had equal identity with the porcine strain YM also. Both the strains differed from RMC321 NSP5 in only 3 amino acids, one difference being common to both. Mc345 and Mc323 are two human isolates from Thailand that had unusual porcine characteristic in their gene constellation as per hybridization studies and sequence analysis [27, 40]. As mentioned earlier, RMC321 also had the same G and P combination (G9P [19]) of Mc345.

Although there are numerous examples of rotavirus strains that appear to have been derived through interspecies transmission, there are few documented evidences where the transmission event has involved a whole virus gene constellation [29]. In spite of the porcine nature of its VP4, VP6, NSP1, NSP3 and NSP4, RMC321 caused severe diarrhoea in a human host. This indicates that, though cross species transmission from porcine to human is rare, it is occurring in nature and in such cases it may not lose its virulence. Such events and inter species transmissions may distort the clustering according to species of origin as has happened in the case of RMC321 and H1, an equine rotavirus closely related to porcine rotavirus in all the genes sequenced so far [5]. The involvement of strains with porcine nature in infantile gastroenteritis indicates that there is a constant flux of genetic material among co-circulating human and animal strains. Routine surveillance and sequencing of VP7 and VP4 genes of rotavirus strains with common and uncommon G/P combinations in UK has revealed that reassortment contributes significantly to overall genetic diversity of rotaviruses [17]. El-Attar et al. [9] experimentally illustrated that rotaviruses circulating in one species pose a risk to another species. Pigs are an integral part of rural life in certain parts of India and hence chances of co-infection with porcine rotaviruses are high. Therefore simultaneous surveillance of animal and human rotavirus strains are essential for a better understanding of the relationship between co-circulating strains [17].

Porcine rotaviruses are more related to human rotaviruses in their VP4, VP6, NSP1, NSP3 and NSP4 [8, 33]. The fact that pigs are more susceptible to human rotaviruses than any other species suggests that the products of at least some of these genes are determinants of host range restriction exhibited by mammalian group A rotaviruses [33]. This genetic similarity between porcine and human strains may help the reassortant strains to sustain virulence in both species. Transmission of animal rotaviruses into humans might occur frequently in developing countries where people live in close proximity to animals under poor sanitary conditions [40]. Manipur is a small state in Eastern India, bordering Myanmar. Most of the people are farmers and many of them live in close proximity to animals, especially pigs. Pork is a favorite dish among tribal people and they rear pigs for food. This condition, together with the poor hygienic practices increases the chances of co-infection with human and animal strains. Understanding whether epizootic infections occur is an important consideration with respect to the attempts to control rotavirus disease by vaccination [29]. Only sequence analysis of the remaining genes will reveal the overall relationship of the genomic constellation of this human rotavirus to porcine strains. Although we

collected porcine stool samples as animal contacts in households with diarrhoeic children during rotavirus surveillance work at Imphal from 1989 to 1993, we did not detect any porcine rotavirus by RNA electrophoresis. None of the contact porcine samples were diarrhoeic. However, with this study, the need for sequence analysis of porcine strains circulating in this region also has gained importance. This study indicates that animal rotaviruses may cross species barrier and cause severe gastroenteritis in humans. Moreover, it also implies the difficulties ahead for developing a successful vaccine to control rotavirus diarrhoea in children.

Acknowledgements

The research was funded by Indian Council of Medical Research (ICMR), Government of India and Japan International Co-operation Agency (JICA), Government of Japan. V. Varghese and S. Das were supported by Senior Research Fellowships from Indian Council of Medical Research and Council of Scientific and Industrial Research, Government of India respectively.

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