

# Full genomic analysis and possible origin of a porcine G12 rotavirus strain RU172

S. Ghosh · N. Kobayashi · S. Nagashima ·  
M. Chawla-Sarkar · T. Krishnan · B. Ganesh ·  
T. N. Naik

Received: 17 November 2009 / Accepted: 27 January 2010 / Published online: 16 February 2010  
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**Abstract** Human group A rotavirus (GAR) G12 strains are regarded as potentially important pathogens for acute gastroenteritis. On the other hand, to date, the only report of detection of G12 in animals was that of a porcine G12P[7] strain RU172. Strain RU172 formed a separate G12 lineage, distinct from human G12 strains, and by analyses of deduced amino acid sequences, had a VP4, VP6, NSP4-5 of porcine origin. In the present study, we determined the full-length nucleotide sequences of VP1, VP3, and NSP1-3 genes and nearly full-length nucleotide sequence of VP2 gene of RU172. By nucleotide sequence identities and phylogenetic analyses, the VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5 genes of RU172 were assigned to G12-P[7]-I5-R1-C1-M1-A1-N1-T1-E1-H1 genotypes, respectively. Within their respective genotypes, (i) VP1 gene of RU172 exhibited higher genetic

relatedness to Wa-like human G12 GARs than porcine strains, (ii) VP2-3 and NSP2 genes clustered separately from the Wa-like human (including G12) and porcine clusters, while (iii) the VP6, NSP1 and NSP3-5 genes clustered with porcine and porcine-like human strains. These observations suggested that (i) the porcine G12 strain might have originated from porcine–human reassortment events, or alternatively, (ii) the Wa-like human and porcine G12 strains might have originated from a common ancestor, and eventually evolved (by genetic drift and shift) with time. Our findings provided important insights into the possible patterns of evolution of the porcine G12 strain.

**Keywords** Group A rotavirus · Porcine strain · G12 genotype

**Electronic supplementary material** The online version of this article (doi:10.1007/s11262-010-0454-y) contains supplementary material, which is available to authorized users.

S. Ghosh (✉) · N. Kobayashi  
Department of Hygiene, Sapporo Medical University  
School of Medicine, S 1, W 17, Chuo-Ku, Sapporo,  
Hokkaido 060-8556, Japan  
e-mail: souvikrota@gmail.com; souvik8@rediffmail.com

S. Nagashima  
Division of Virology, Department of Infection and Immunity,  
Jichi Medical University School of Medicine,  
Tochigi-ken, Japan

M. Chawla-Sarkar · T. Krishnan · B. Ganesh  
Division of Virology, National Institute of Cholera and  
Enteric Diseases, Kolkata, India

T. N. Naik  
School of Biology, National Institute of Science Education  
and Research, Bhubaneswar, India

## Introduction

Group A rotaviruses (GAR) are major causes of severe diarrhea in the young ones of humans and animals [1]. The GAR genome consists of 11 segments of double-stranded RNA that encode six structural and six nonstructural proteins [1]. Among the structural proteins, VP4 and VP7 constitute the outer layer of the viral capsid, VP6 forms the inner capsid, VP2 is the major inner core protein, VP3 functions as the RNA capping enzyme, and VP1 is an RNA-dependent RNA polymerase [1]. The nonstructural proteins play significant roles in virus replication and morphogenesis [1].

The GAR outer capsid proteins, VP4 and VP7, elicit the production of neutralizing antibodies, and therefore, information on diversity of these genes are important for vaccine development [1]. To date, GARs have been classified into at

least 23 G and 31 P genotypes on the basis of differences in their VP7 and VP4 gene sequences, respectively [2–4]. Among them, G1, G2, G3, G4, and G9 in conjunction with P[4], P[6], and P[8] are commonly found in humans [5–8]. In addition to these, recent surveillance studies have identified G12 as a globally important genotype in humans [9–24]. The first human G12 strain was reported from the Philippines in 1987 [25]. Thereafter, almost a decade later, human G12 strains were detected in Thailand [26]. In subsequent years, human G12 strains were reported from several countries, linked to high rates of childhood diarrhea in countries like India, Nepal, and Bangladesh, and detected in association with multiple VP4 genotypes [2, 9–24]. Therefore, G12 strains are now being recognized as potentially important human pathogens.

Unlike in humans, the only report of detection of G12 in animals was that of a single porcine G12P[7] strain, RU172, from a 1-month-old diarrheic piglet at a farm in eastern India [27]. In spite of its G12 genotype nature (>90% deduced amino acid (aa) identities to human G12 strains), the VP7 gene of strain RU172 appeared to be distinct from human G12 strains [27]. Among the other genes analyzed at deduced aa level, RU172 had a VP4 (P[7]), VP6, NSP4 and NSP5 of porcine origin [27]. The detection of RU172 and identification of a porcine-like NSP2 in a human G12 strain (L26) raised speculations as to whether human G12 strains originated from porcine GARs [16, 27]. Therefore, information on the remaining six gene segments (VP1–3 and NSP1–3) of porcine G12 strain RU172 are essential to decipher the exact genetic relatedness between porcine and human G12 GARs.

In the present study, we determined the full-length nucleotide (nt.) sequences of VP1, VP3, NSP1, NSP2, and NSP3 genes and nearly full-length nt. sequence of VP2 gene of the porcine GAR G12 strain RU172. The full genome of RU172 was analyzed at nt. level following the scheme proposed by the Rotavirus Classification Working Group (RCWG) [2, 28]. In order to properly understand the origin of G12 genotype, we studied the genetic relatedness of full genome of porcine G12 strain RU172 to those available for human G12 strains. Our findings provided important insights into the possible patterns of evolution of G12 rotaviruses.

## Materials and methods

### Viruses

Porcine GAR G12P[7] strain RU172 was detected in a 1-month-old diarrheic piglet at a farm located near the city of Kolkata, eastern India [27]. The fecal sample was collected during the month of February of 2002.

### RT–PCR and sequencing

For RT–PCR, viral RNA was extracted from the stool sample using the QIAamp Viral RNA Mini kit (Qiagen Sciences, MD, USA). Primers used for the amplification of VP1, VP2, VP3, NSP1, NSP2, and NSP3 genes of porcine G12 strain RU172 were designed from conserved stretches of cognate genes of several published GAR strains (Supplementary table S1). In the present study, the full-length nt. sequence of VP2 gene of RU172 could not be determined. Repeated attempts with end primers designed from several published strains failed to amplify the 3′-portion of VP2 of RU172. Nevertheless, using a primer more upstream, we could sequence a major stretch (2647 bp) of VP2 gene of strain RU172. Several internal primers, designed from partial sequences of RU172, were used in RT–PCR assays to complete the full-length nt. sequence of VP1 and nearly full-length nt. sequence of VP2 genes (Supplementary table S1). The RT–PCR conditions were identical to those described previously [17, 29]. Nucleotide sequences were determined using the BigDye Terminator v3.1 Cycle Sequencing Reaction kit (Applied Biosystems, CA, USA) on an automated sequencer (ABI PRISM 3100).

### Sequence analysis

Sequence comparisons were carried out as described previously [27]. Phylogenetic trees were constructed by the neighbor-joining method [30] using the MEGA software (version 4.1). Bootstrap analysis was performed based on 1000 replicates, and phylogenetic distances were measured by the Kimura two-parameter model. Genotypes were assigned to the eleven gene segments of strain RU172 following the RCWG classification scheme [2, 28]. For a given gene segment, published strains with full-length, nearly full-length or complete ORF (open reading frame) nt. sequences were only included in the analysis.

### Nucleotide sequence accession numbers

The nt. sequences of VP1, VP2, VP3, NSP1, NSP2, and NSP3 genes of porcine GAR G12P[7] strain RU172 were submitted to the GenBank database, and assigned consecutive nt. sequence accession numbers GU199191–GU199196.

## Results and discussion

Full genome analyses of GARs are important to obtain conclusive data on the origin of a strain and its genetic relatedness to other strains [28]. The recently introduced RCWG classification scheme, based on full genome analysis of GARs, provided an excellent platform to trace the

origin of unusual strains, especially those derived from interspecies reassortment events [2, 28]. Moreover, unlike previous studies, the RCWG scheme favored nucleotide sequences to deduced aa sequences, as results were found to be reliable at nt. level than at the aa level [2, 28]. The results obtained using this scheme were in agreement with the broader classification of GARs into the three major GAR genogroups (Wa, DS-1, and AU-1) [2]. Therefore, following the RCWG classification scheme, we analyzed

the VP1, VP2, VP3, NSP1, NSP2, and NSP3 genes and reanalyzed the VP6, NSP4, and NSP5 genes of the only reported animal GAR G12 strain RU172. By nt. sequence identities and phylogenetic analyses, the VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5 genes of porcine G12P[7] strain RU172 were assigned to G12-P[7]-I5-R1-C1-M1-A1-N1-T1-E1-H1 genotypes, respectively (Table 1; Fig. 1a–f). The R1, C1, M1, A1, N1, T1, E1, and H1 genotypes also include the human GAR strain Wa,

**Table 1** Nucleotide sequence identities (%) of VP1-4, VP6-7, and NSP1-5 genes of porcine group A rotavirus (GAR) G12 strain RU172 to those of human G12 and other porcine GAR strains

Strain	Nucleotide sequence identities (%)										
	VP1	VP2	VP3	VP4	VP6	VP7	NSP1	NSP2	NSP3	NSP4	NSP5
Porcine strains <sup>a</sup>	84.3-86.2	75.4-76.5	85.3-87.4	63.2-92.1	82.8-93.1	73.1-76.7	78.5-95.0	82.8-88.4	83.6-89.9	82.7-100	90.1-97.8
<b>Human G12 strain</b>											
Dhaka25-02	89.0	87.0	86.2	65.1	83.9	90.1	82.5	88.9	87.8	89.4	97.3
Dhaka12-03	89.2	87.2	86.4	63.4	83.7	89.6	82.5	87.9	87.2	89.1	97.1
B4633-03	89.0	86.9	86.1	65.2	84.1	89.9	82.5	88.2	87.3	89.4	96.7
Matlab 13-03	88.9	86.8	86.5	63.5	83.7	89.6	82.6	88.7	79.6	89.1	97.1
GER126-08	88.2	87.7	85.6	64.7	82.8	89.6	81.8	88.1	88.2	90.3	97.1
GER172-08	88.1	87.3	86.0	63.2	83.7	89.4	81.2	88.0	86.7	89.1	96.3
SK277	<sub>b</sub>	<sub>b</sub>	<sub>b</sub>	63.5	83.9	90.4	82.0	88.1	87.3	89.4	95.9
US6588	<sub>c</sub>	<sub>c</sub>	<sub>c</sub>	64.9	83.9	89.9	81.8	<sub>c</sub>	<sub>c</sub>	89.1	96.2
US6597	<sub>c</sub>	<sub>c</sub>	<sub>c</sub>	63.0	83.7	89.6	82.0	<sub>c</sub>	<sub>c</sub>	89.6	96.0
L26	79.4	79.8	85.8 or 75.9 <sup>d</sup>	64.9	80.1	91.8	76.0	88.6	80.3	82.1	97.3
RV161-00	79.2	79.4	76.8	63.5	79.5	89.9	75.9	82.6	79.5	89.3	81.0
RV176-00	79.1	79.4	76.8	63.5	79.5	89.9	75.9	82.7	79.4	82.8	81.1
N26-02	79.2	79.4	76.8	62.8	79.8	89.9	76.1	88.6	79.3	83.5	80.3
T152	79.6	80.2	77.6	62.9	77.7	89.8	62.1	81.4	79.1	82.1	88.2
<b>Reference strain</b>											
Wa	89.0	88.9	87.3	65.0	83.3	76.3	81.1	88.1	88.1	90.0	92.6
DS-1	78.7	79.3	77.0	65.1	79.9	74.9	75.4	79.6	78.0	82.4	82.0
AU-1	80.1	79.8	77.9	61.6	77.4	75.8	66.0	80.8	77.9	81.4	87.7

Reference strains representing the three major GAR genogroups were also included in the analysis

The Wa-like, DS-1-like and AU-1-like genes are shown in light, medium and dark shades of grey, respectively

<sup>a</sup> Porcine group A rotavirus strains included in the analysis were A131, A253, Gottfried and YM for VP1; A131 and A253 for VP2; A131, A253, OSU and YM for VP3; 134/04-15, 34461-4, 4F, Gottfried, HP140, JL94, OSU, P21-5, P343, S8 and YM for VP4; 4F, 4S, A131, A253, A411, CMP034, CN86, CRW-8, Gottfried, OSU and YM for VP6; A131, CMP034, Gottfried, HP140, OSU, P21-5, P343 and YM for VP7; 4F, 4S, A131, A253, A411, Gottfried, OSU and YM for NSP1; A131, A253, A411 and OSU for NSP2; A131, A253, A411, GO, Gottfried and OSU for NSP3; 221/04-7, 221/04-13, 221/04-21, 344/04-1, A2, A34, A131, A253, A411, ES51/04, CMP034, HP113, HP140, OSU and YM for NSP4; and 134/04-11, A131, A253, CC86, C60, CMP034, CN86, HP113, HP140, OSU and YM for NSP5 genes, respectively

<sup>b</sup> No sequence data were available for VP1-3 genes of strain SK277 in the GenBank database

<sup>c</sup> Short stretches (<300 bp) of nucleotide sequences were available for VP1-3 and NSP2-3 genes of strains US6588 and US6597 in the GenBank database, and therefore, these were not included in the present study. These genes are Wa-like [15]

<sup>d</sup> Two different nucleotide sequences (Wa-like and DS-1-like) with accession numbers AY277918 and EF583035 were available for VP3 gene of strain L26 in the GenBank database

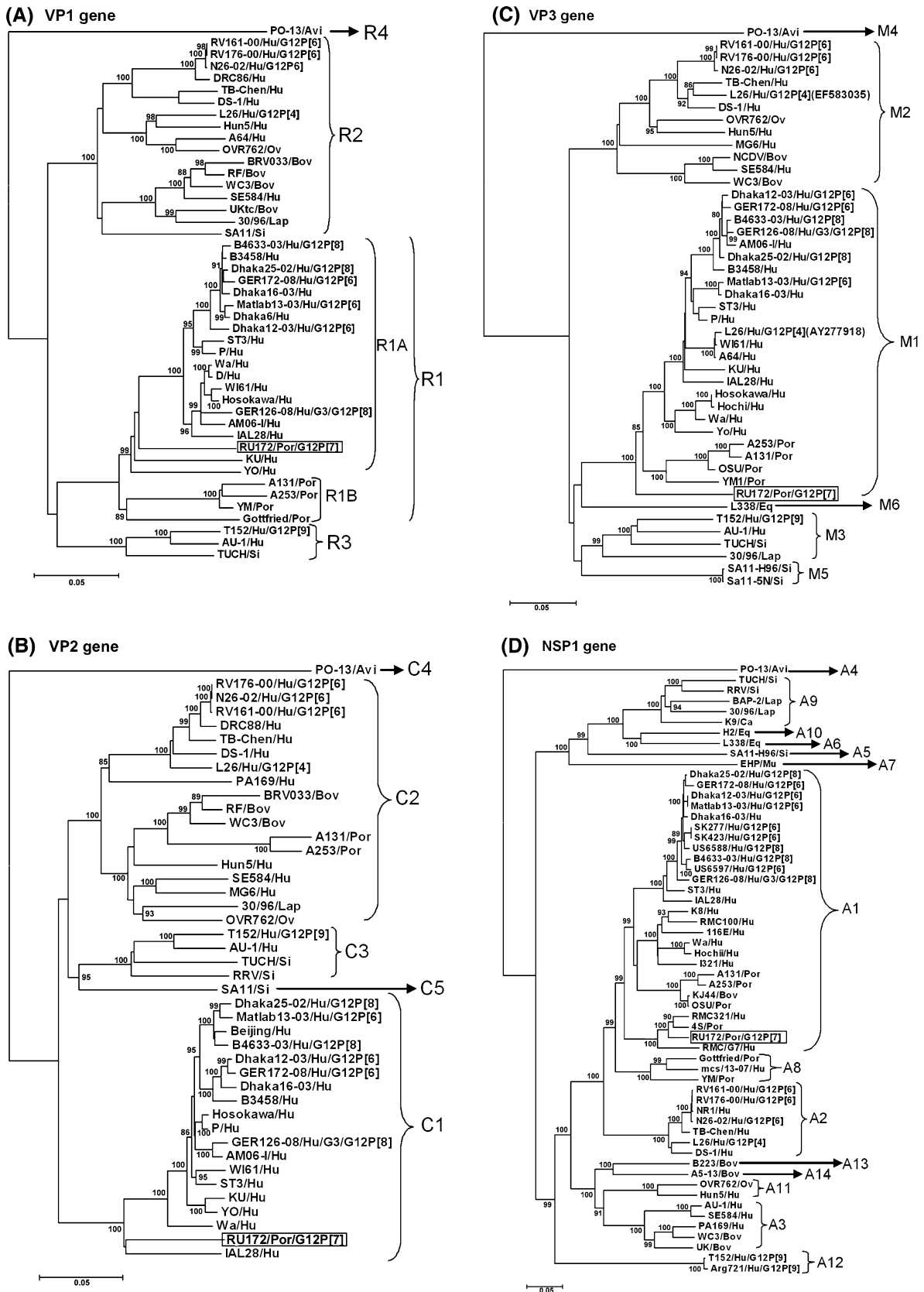
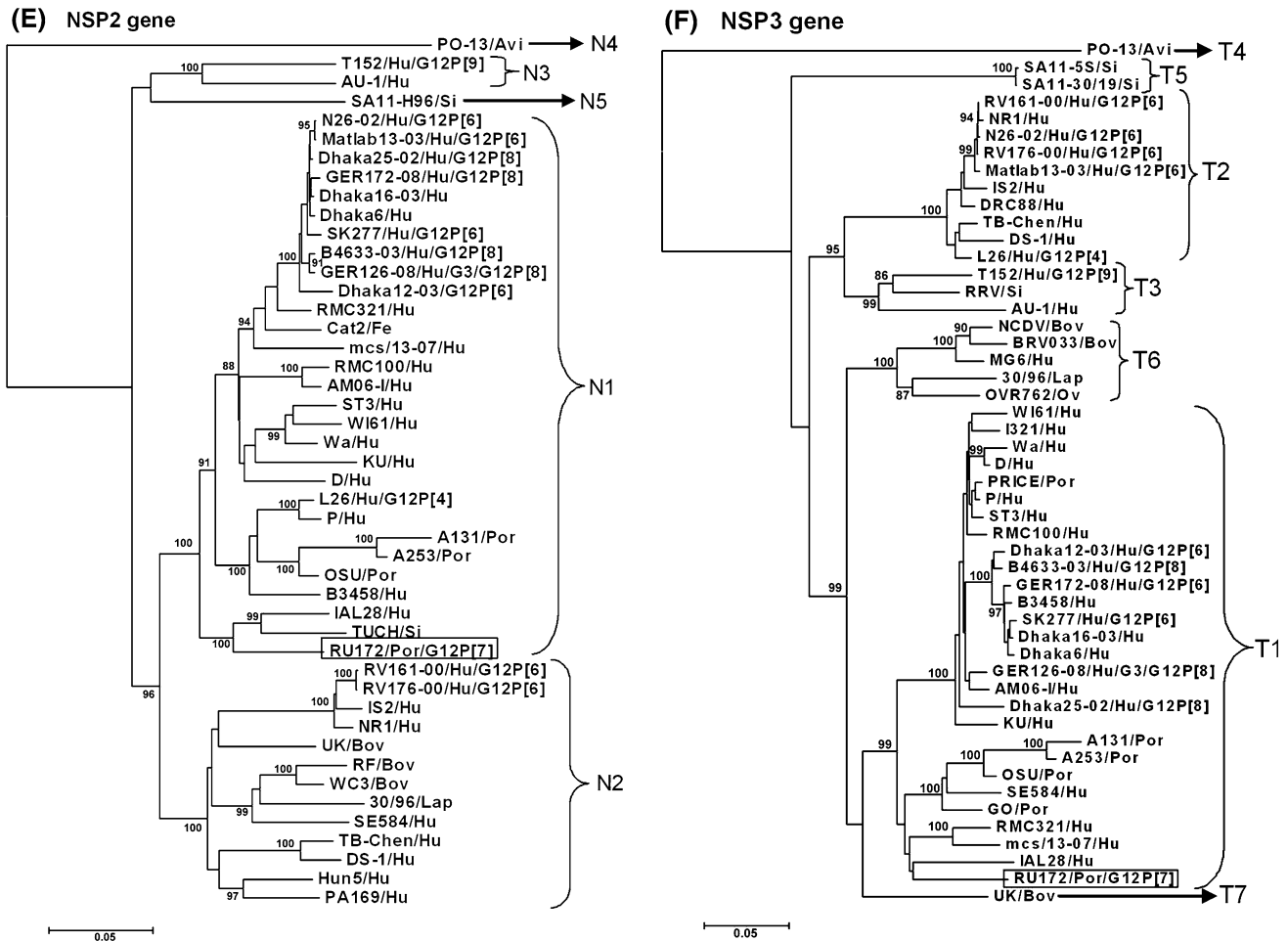


Fig. 1



**Fig. 1** a–f Phylogenetic trees constructed from nucleotide sequences of VP1, VP2, VP3, NSP1, NSP2, and NSP3 genes of porcine G12P[7] strain RU172 with those of group A rotavirus strains representing the 4 R, 5 C, 6 M, 14 A, 5 N and 7 T genotypes, respectively. In Fig. 1a. The two major clusters, R1A and R1B, within genotype R1 are

shown. In all the trees, strain RU172 is boxed. The G-P combinations of G12 strains are indicated. Bootstrap values  $\geq 85\%$  are shown. Bar, 0.05 substitutions per nucleotide. *Abbreviations:* *Avi* avian, *Bov* bovine, *Ca* canine, *Eq* equine, *Fe* Feline, *Hu* human, *Lap* lapine, *Mu* Murine, *Ov* ovine, *Por* porcine, and *Si* simian

Wa-like human strains including G12 GARs and porcine strains, while the I5 genotype consisted of porcine and porcine-like human strains [2, 15, 22].

By nt. sequence identities, it was difficult to ascertain as to whether porcine G12 strain RU172 was more related to Wa-like human G12 or other porcine strains. The VP1 gene of strain RU172 shared higher nt. identities with those of Wa-like human G12 strains than porcine strains (Table 1), while the NSP1, NSP4, and VP6 genes exhibited higher identities to those of porcine strains (Table 1). On the other hand, VP3, NSP3, and NSP5 genes of RU172 exhibited significant nt. sequence identities to those of Wa-like human (including G12) and porcine strains (Table 1). The VP2 gene of RU172 exhibited higher nt. identities to those of Wa-like human (including G12) strains than to the only available full-length porcine VP2 sequences of strains A131 and A253 (Table 1). However, surprisingly, the VP2

genes of A131 and A253 clustered with bovine GARs within genotype C2 (Fig. 1b). Interestingly, the NSP2 gene of RU172 exhibited a high nt. identity of 89.4% to simian strain TUCH, though maximum identity of 91.4% was observed with the porcine–human reassortant strain IAL28. The NSP2 gene of RU172 shared significant nt. identities with those of Wa-like human (including G12) and porcine strains (Table 1).

Phylogenetic analyses of the VP1-3, VP6, and NSP1-5 genes of RU172 revealed its overall genetic relatedness to Wa-like human (including G12) and porcine strains. Within their respective genotypes, (i) the VP1 gene of RU172 clustered with Wa and Wa-like human GARs including G12 strains within cluster R1A, away from the porcine cluster (R1B) (Fig. 1a), (ii) the VP6, NSP1, NSP3, NSP4, and NSP5 genes clustered with porcine and porcine-like human strains (Fig. 1d, f, data on VP6, NSP4,

and NSP5 genes are not shown as the clustering patterns of RU172 were identical to those reported by Matthijnssens et al. 2008 [2]), while (iii) the VP2, VP3, and NSP2 genes clustered separately from the Wa-like human and porcine clusters (Fig. 1b, c, e).

G12 strains are being frequently encountered in humans [9–24], while, to date, a single porcine G12 strain has been reported [27]. Interestingly, none of the human G12 strains were reported in conjunction with the P[7] VP4 genotype, common in porcine GARs. Therefore, with the present available information, the possibility that G12 is a common human genotype and that the porcine G12 strain is a rare instance of reassortment events involving Wa-like human G12 and porcine P[7] strains cannot be ruled out. However, surveillance studies on porcine GARs are limited, and therefore, it might be too premature to assume that G12 is a rare porcine genotype. Nevertheless, (i) presence of a Wa-like human VP1 in RU172 and (ii) a porcine-like NSP2 in human G12 strain L26 [16] indicate that the G12 strains might have originated from human–porcine reassortment events.

Although the VP1 gene of RU172 exhibited higher genetic relatedness to Wa-like human GARs including G12 strains than other porcine strains, the clustering pattern indicated that the RU172 VP1 might not have evolved recently. This observation was supported by the clustering patterns of VP2, VP3, and NSP2 genes of RU172. The VP7 gene of RU172 formed a separate lineage, distinct from human G12 strains [27]. On the other hand, the VP4 (VP8\*) [27], VP6, NSP1, NSP3, NSP4, and NSP5 genes of RU172 were more related to porcine strains than Wa-like human G12 strains. Although RU172 had G12P[7] specificity, many human G12 strains were reported in conjunction with P[6] [9–24], a VP4 genotype also commonly found in pigs [1, 2, 31]. Taken together, these observations suggest that the porcine and Wa-like human G12 strains might have originated from a common ancestor long time back, and eventually the porcine strains, like their human counterparts, might have evolved (by genetic drift and shift) with time. The VP1 gene of RU172 might have retained the genetic evidence in support of this hypothesis.

In conclusion, the present study provided important insights into the genetic relatedness between human and porcine G12 strains. However, due to dearth of information on other animal (porcine) G12 strains, we could not ascertain the origin of this genotype. On the other hand, in humans, as of now, full genome sequences are available for only 13 of the several G12 strains detected worldwide [15, 16, 22]. Full genome analyses of these strains have already revealed the vulnerability of human G12 strains to frequent reassortment events within and between genogroups [15, 16, 22]. Therefore, comparative analyses of full genome sequences of several human and animal G12 strains are required to pinpoint the true origin of this emerging genotype.

**Acknowledgments** The study was supported in part by the grant-in-aid for scientific research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Grant number 20-08463), Program for developing the supporting system for upgrading the education and research, and Program of Founding Research Centers for Emerging and Reemerging Infectious Diseases (Okayama University–National Institute of Cholera and Enteric Diseases, India).

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