Virus Genes (2007) 35:141–145
DOI 10.1007/s11262-007-0089-9

# Molecular identification of a novel G1 VP7 gene carried by a human rotavirus with a super-short RNA pattern

**Kamruddin Ahmed · Toyoko Nakagomi · Osamu Nakagomi**

Received: 19 July 2006 / Accepted: 20 February 2007 / Published online: 16 March 2007
© Springer Science+Business Media, LLC 2007

**Abstract** AU19 is a human rotavirus strain carrying a G1P2C[6] specificity and a super-short RNA pattern. Neutralization assay revealed an asymmetric cross neutralization between AU19 and Wa, a representative G1 rotavirus strain, and it is noteworthy that AU19 was neutralized poorly by anti-Wa hyper-immune serum. In the phylogenetic tree the VP7 gene of AU19 formed a lineage different from the established lineages of known human G1 rotaviruses. A signature code MFTKLLTAA was noted for AU19 lineage. These results provide evidences that the VP7 gene of AU19 represents a novel G1 lineage.

**Keywords** Rotavirus · Serotype G1P2C[6] · Super-short RNA pattern

## Introduction

Group A rotavirus is generally responsible for the majority of infantile diarrhea in humans. Based on the diversity of VP7 and VP4 antigens present on the outer capsid, rotavirus is classified into 15 G and 26 P types [1]. Among them G1, G2, G3, G4, and G9 types are mainly associated with human infection throughout the world [2]. Therefore the target of current rotavirus vaccination strategy is to prevent infection caused by those serotypes.

The unique arrangement of rotavirus genome into 11 segments of dsRNA generally produces a pattern called long RNA pattern when separated by polyacrylamide gel electrophoresis. In some strains, mainly in serotype G2 strains, genetic rearrangement occurs in segment 11 resulting in a slower electrophoretic migration of this segment than segment 10, which causes a pattern called short RNA pattern [3–5]. In rare occasions, rearranged segment 11 becomes larger which migrates further slowly during electrophoresis and causes a pattern called super-short RNA pattern. Super-short RNA pattern is rarely found in human rotaviruses. AU19 is the first rotavirus strain isolated in Japan that has a super-short RNA pattern [6]. Although it is a serotype G1 strain, AU19 has a P serotype designated as P2C[6] that is different from either serotype P2A[6] carried by asymptomatic neonatal strains or P2B[6] carried by porcine rotavirus strain Gottfried [6]. Both VP7 and VP4 antigens elicit neutralizing antibodies; the antibodies against VP7 play a greater role than antibodies against VP4 for immunity after natural rotavirus infections [7] as well as after vaccination [8]. Thus, VP7 diversity has significant implications on rotavirus vaccination. Therefore the present study was undertaken to further characterize the VP7 of AU19 by examining its VP7 gene to elucidate whether this type of strain may pose a challenge as vaccine escape strain.

The detection and isolation of AU19 were described previously [6]. Genomic dsRNA was extracted by phenol–chloroform–isoamyl alcohol from partially purified virions prepared by pelleting the infected culture supernatant [9]. Standard plaque reduction cross neutralization assay was done on AU19 and Wa by using hyper-immune sera raised in guinea pigs against AU19 and Wa, respectively.

Amplification by reverse transcription (RT)-PCR of the rotavirus VP7 gene was performed as described previously [10]. The PCR product was purified using Wizard PCR Preps DNA purification System (Promega Co., Madison, WI, USA) according to the instructions by the manufacturer.

K. Ahmed · T. Nakagomi · O. Nakagomi (✉)
Division of Molecular Epidemiology, Department of Molecular Microbiology and Immunology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki 852-8523, Japan
e-mail: onakagom@nagasaki-u.ac.jp

**Table 1** Serological characterization of AU19 by the plaque reduction neutralization assay

| Virus | Serotype | Reciprocal of neutralization titer of antiserum to | |
|---|---|---|---|
| | | AU19 | Wa |
| AU19 | G1P2C[6] | 102,400 | 594 |
| Wa | G1P1A[8] | 6,400 | 40,892 |

Nucleotide sequence was determined by dideoxy chain termination method using ABI PRISM Dye terminator cycle sequencing ready reaction kit (Perkin-Elmer, Foster City, CA, USA) and an ABI PRISM 310 genetic analyzer (Perkin-Elmer).

In order to examine the relationships with other rotavirus strains, nucleotide sequence alignment was carried out using CLUSTAL X, version 1.82 [11] and a phylogenetic tree was constructed based on deduced amino acid sequences by using the neighbor-joining method with bootstrap analysis of 1,000 replicates [12]. *N*-glycosylation sites on the amino acid sequence of VP7 were predicted by NetNGlyc 1.0 Server.

**Table 2** Percent identity of amino acid and nucleotide sequences of VP7 genes of AU19 and selected rotavirus strains

| Strain (origin) | G type | nt identity (%) | aa identity (%) |
|---|---|---|---|
| AU64 (human) | 1 | 86 | 91 |
| AU007 (human) | 1 | 87 | 93 |
| Wa (human) | 1 | 86 | 91 |
| M37 (human) | 1 | – | 92 |
| C60 (porcine) | 1 | 85 | 93 |
| T449 (bovine) | 1 | 85 | 92 |
| KUN (human) | 2 | 73 | 73 |
| SA11 (simian) | 3 | 75 | 81 |
| Gottfried (porcine) | 4 | 74 | 78 |
| OSU (porcine) | 5 | 77 | 79 |
| UK (bovine) | 6 | 74 | 83 |
| PO-13 (avian) | 7 | 64 | 57 |
| A5 (bovine) | 8 | 73 | 77 |
| 116E (human) | 9 | 75 | 79 |
| KK3 (bovine) | 10 | 76 | 79 |
| YM (porcine) | 11 | 75 | 80 |
| L26 (human) | 12 | 73 | 75 |
| L338 (equine) | 13 | 74 | 75 |
| CH3 (equine) | 14 | 76 | 78 |
| Hg18 (bovine) | 15 | 71 | 76 |

The VP7 nucleotide sequences used were from the following accession numbers: for AU19, AB018697; for AU64, AB081801; for AU007, AB081799; for Wa, K02033; for M37, P11852; for C60, L24164; for T449, M92651; for KU, D16343; for KUN, D50124; for SA11, V01546; for Gottfried, X06386; for OSU, X04613; for UK, X00896; for PO-13, D82979; for A5, D01054; for 116E, L14072; for KK3, D01056; for YM, M23194; for L26, M58290; for L338, D13549; for CH3, D25229; and for Hg18, AF237666. The nucleotide sequence of the VP7 gene of M37 is unavailable at the GenBank

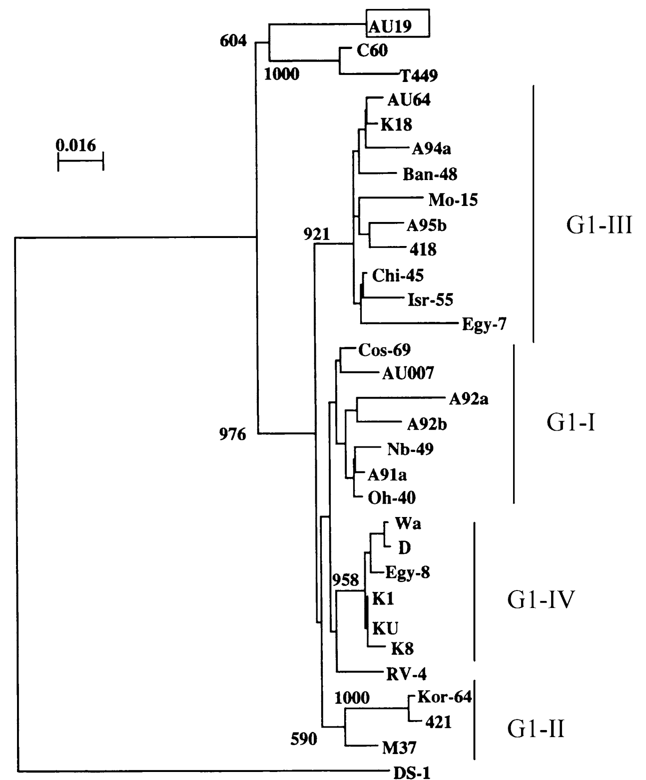


**Fig. 1** Phylogenetic tree constructed from the deduced amino acid sequences of the VP7 genes of animal and human G1 rotaviruses. DS-1, a human G2 strain, was used as an out-group. The VP7 gene of strain AU19 segregated into a lineage different from conventional lineages (G1-I, G1-II, G1-III and G1-IV) of human G1 strains. Closely related to AU19, strains C60 and T449 are porcine and bovine G1 strains, respectively

As shown in Table 1, anti-AU19 hyper-immune serum neutralized Wa at a titer 16-fold lower than the homologous neutralization titer, while anti-Wa hyper-immune serum neutralized AU19 at a titer less than 64-fold lower than the homologous neutralization titer. This indicated that the cross-neutralization relationship between AU19 and Wa viruses was asymmetrical. This asymmetric relationship between AU19 and Wa imply that although AU19 belongs to serotype G1, there is immunogenic differences between the two strains.

Nucleotide and deduced amino acid sequence analyses of the VP7 gene showed that AU19 had 86–87% nucleotide and 91–93% amino acid identities with strains of lineage I, II, III and IV of G1 rotaviruses represented by AU007, M37, AU64 and Wa, respectively (Table 2). It had 85% nucleotide and 92–93% amino acid identities with porcine and bovine G1 rotavirus strains C60 and T449, respectively. When the VP7 antigenic regions A (aa 87–101), B (aa 143–152), C (aa 208–223) and F

```
                 1                                                  50
AU007G1-I   MYGIEYTTIL IFLISIILLN YILKSVTRMM DYIIYRSLLI SVTLFALTRA
AU64  G1-II .......... .......... ........I. ......F... ..A.....K.
M37   G1-III .......... .....V.... ........I. ......F... T.A.......
Wa    G1-IV .......... .......... ........I. ......F... T.A.......
AU19  G1-N  .......... .....V..F. .......... ......F... TM...V.AK.
T449  G1-O  .......... T......... .....T.Q.. ......F... T.......K.
C60   G1-O  .......... T......... .....T.Q.. ......F... T.......K.

            51                                                 100
                                                       Region A
AU007G1-I   QNYGLNLPIT GSMDTAYANS TQEGIFLTST LCLYYPTEAS TQINDGEWKD
AU64  G1-II ......I... .....V.S.. ....V..I.. .......... ...S......
M37   G1-III .......... .....I.... .R........ .......... ...S......
Wa    G1-IV .......... ....AV.T.. ...EV..... .......... ......D...
AU19  G1-N  .......... .......... ...ET..... ......I... N.........
T449  G1-O  ....I..... .......... .K.ET.M... ......V... N.........
C60   G1-O  ....I..... .......... .K.ET.M... ......V... N.........

            101                                                150
                                                        Region B
AU007G1-I   SLSQMFLTKG WPTGSVYFKE YSSIVDFSVD PQLYCDYNLV LMKYDQNLEL
AU64  G1-II .......... .......... ..N....... .......... ..........
M37   G1-III .......... .......... .......... .......... ..........
Wa    G1-IV .......... .......... ..N....... .......... ......S...
AU19  G1-N  T......... .......... .T...E.... .......... ..........
T449  G1-O  T......... .......... .TN....... .......... ..........
C60   G1-O  T......... .......... .TN....... .......... ..........

            151                                                200

AU007G1-I   DMSELADLIL NEWLCNPMDI TLYYYQQSGE SNKWISMGSS CTVKVCPLNT
AU64  G1-II .......... .......... ........E. .......... ..........
M37   G1-III .......... .......... .......... .......... ..........
Wa    G1-IV .......... .........V .......... .......... ..........
AU19  G1-N  .......... .......... K......A.. .......... ..........
T449  G1-O  .......... .......... .......T.. .......... ..........
C60   G1-O  .......... .......... .......T.. .......... ..........

            201                                                250
                        Region C                  Region F
AU007G1-I   QTLGIGCQTT NVDSFEMVAE NEKLAIVDVV DGINYKINLT TTTCTIRNCK
AU64  G1-II .......... ......T... .......... ....H..... ..........
M37   G1-III .......R.. .......... .......... ....H..... ..........
Wa    G1-IV .......... .......I.. .......... ....H..... ..........
AU19  G1-N  .......... ......I... D......... ....H..... ..........
T449  G1-O  .......... QCG...I... .......... ....H..... ..........
C60   G1-O  .......... ......I... .......... ....H..... ..........

            251                                                300
AU007G1-I   KLGPRENVAV IQVGGSNVLD ITADPTTNPQ TERMMRVNWK KWWQVFYTIV
AU64  G1-II .......... .......I.. .......... I......... R.........
M37   G1-III .......... .......... .......... .......... R.........
Wa    G1-IV .......... .......... .......... .......... ..........
AU19  G1-N  .......... .......I.. .......... .......... ..........
T449  G1-O  .......... .......I.. .......... I..I...... E..K......
C60   G1-O  .......... .......I.. .......... I......... ..........
             301                      326

AU007 G1-I   DYINQIVQVM SKRSRSLNSA AFYYRV
AU6   G1-II  .......... .......... ......
M37   G1-III .......... .......... ......
Wa    G1-IV  .......... .......... ......
AU19  G1-N   ......I... .......... ......
T449  G1-O   .......... .......... G.....
C60   G1-O   ........L. .......... ......
```

**Fig. 2** The lineage signature codes and the antigenic regions of VP7 of AU19 are compared with that of different G1 lineages. Strain names are followed by G1 lineage names, G1-N and G1-O indicates new and other lineage, respectively. Lineage specific amino acid residues 29, 37, 41, 49, 55, 57, 65, 66 and 68 are highlighted with halftone. The antigenic regions A (amino acid 87–101), B (aa 143–152), C (aa 208–223) and F (aa 235–242) are indicated with overlines

(aa 235–242) [13, 14] of AU19 were compared with those of the above representative strains of the four lineages of human G1 rotaviruses, unique substitutions were found in region A, 87 (T → I), 91 (T → N), 101 (S → T); and region C, 217 (M or T → I), 221 (N → D). Especially with the Wa strain additional substitutions were present at amino acid residues 97 (D → E) in region A, at 147 (S → N) in region B and at

218 (I → V) in region C. When the VP7 antigenic regions of AU19 were compared with those of strains C60 and T449, substitutions were observed at 87 (V → I) in region A and at 212 (C → V, same as C60), 213 (G → D, same as C60) and 221 (N → D) in region C.

Phylogenetic analysis of the deduced amino acid sequences of the VP7 genes of G1 strains revealed that two main branches were segregated from the common ancestor of G1 strains at the early stage of evolution (Fig. 1). One of the branches contains the conventional G1 lineages. The other branch contains strains C60 and T449, which formed a group with full bootstrap value support segregating from the AU19 lineage.

Among the G1 strains, a lineage/sub-lineage signature code is present in nine amino acid residues (29, 37, 41, 49, 55, 57, 65, 66 and 68) near the N-terminus of the VP7 [15]. In AU19 this code was MFTKLLTAA which is different from any of the lineage or sub-lineage signature codes of G1 rotaviruses (Fig. 2). Two potential *N*-glycosylation sites were predicted at amino acid residues 69 and 238 on the VP7 of AU19, these are conserved sites for glycosylation in other G1 rotaviruses [16].

The present study showed that like its VP4 gene, the VP7 gene of AU19 has also unique features. The asymmetric and weak neutralization of Wa by anti-AU19 hyper-immune serum might be due to variation in the antigenic determinants or glycosylation [17] of the VP7 of these two G1 rotaviruses. The prediction that glycosylation sites of the VP7 of AU19 are the same as in other G1 rotaviruses [16] supports the view that the basis for the asymmetric neutralization relationship between AU19 and Wa lies in the differences in the antigenic determinants. When the VP7 amino acid sequences of Wa and AU19 were compared, notable variations were found at amino acid resides 87, 217 and 221, which are among the several known sites of substitutions responsible for neutralization escape mutants [18]. These residues are in the antigenic regions A and C which appear to be distantly located on the linear molecule but which are actually brought together in the folded molecule of VP7 [13]. Therefore these residues may play a critical role for neutralization escape in our study.

Phylogenetic analysis revealed that segregation of the VP7 gene of AU19 might have occurred at an early stage of evolution from the contemporary G1 strains which later separated into the well established four lineages [19]. In agreement with the serological and genetic differences between AU19 and the strains of other G1 lineages, phylogenetic analysis provided evidences that AU19 belongs to a new lineage of G1 rotaviruses. This new lineage was supported by the lineage signature code found in this strain. Apparently strains C60 and T449 did not belong to the AU19 lineage. Whether these two strains belong to the AU19 lineage or sublineage needs further investigation. Strains C60 and T449 are porcine and bovine G1 strains, respectively [16, 20] with a VP7 amino acid sequence identity of 97.8% [21]. Bovine and porcine G1 strains are not common but their presence has been documented in some field isolates [22, 23]. Considering all these findings together, we conclude that AU19 represents a novel lineage of G1 rotavirus.

In a recent publication the VP4 genes of two G9P[6] porcine rotaviruses were found to have significant homology with that of AU19 [24]. This suggests that AU19-like strains contribute to the evolution of rotavirus strains in nature. However, the direct impact of this strain in the community is not clear because we did not examine in a systematic fashion whether there was any further circulation of AU19-like strains in the same geographic location. Further studies are also needed to address the relationship of AU19 and porcine rotavirus strains. Genetic reassortment frequently serves as a driving force in rotavirus evolution. One of the challenges to rotavirus vaccines is the emergence of unusual strains. Since we demonstrated that anti-serum generated by standard G1 strain could not neutralize AU19 because of its unique VP7, there is a possibility that AU19-like strains may escape the immunity conferred by vaccines carrying a standard VP7 of G1 specificity.

## References


1. V. Martella, M. Ciarlet, K. Banyai, E. Lorusso, A. Cavalli, M. Corrente, G. Elia, S. Arista, M. Camero, C. Desario, N. Decaro, A. Lavazza, C. Buoavoglia, Virology **346**, 301 (2006)
2. R.I. Glass, U.D. Parashar, J.S. Bresee, R. Turcios, T.K. Fischer, M.-A. Widdowson, B. Jiang, J.R. Gentsch, Lancet **368**, 323 (2006)
3. S.A. Gonzales, N.M. Mattion, R. Bellinzoni, O.R. Burrone, J. Gen. Virol. **70**, 1329 (1989)
4. M. Gorziglia, K. Nishikawa, N. Fukuhara, Virology **170**, 587 (1989)
5. S.M. Matsui, E.R. Mackow, S. Matsuno, P.S. Paul, H.B. Greenberg, J. Virol. **64**, 120 (1990)
6. T. Nakagomi, Y. Horie, Y. Koshimura, H.B. Greenberg, O. Nakagomi, J. Clin. Microbiol. **37**, 1213 (1999)
7. S. Chiba, T. Yokoyama, S. Nakata, Y. Morita, T. Urasawa, K. Taniguchi, S. Uraswas, T. Nakao, Lancet **2**, 417 (1986)
8. A.Z. Kapikian, Y. Hoshino, R.M. Chanock, I. Perez-Schael, J. Infect. Dis. **174**(Suppl 1), S65 (1996)
9. O. Nakagomi, T. Nakagomi, Arch. Virol. **119**, 67 (1991)
10. M. Watanabe, T. Nakagomi, Y. Koshimura, O. Nakagomi, Arch. Virol. **146**, 557 (2001)
11. J.D. Thompson, T.J. Gibson, F. Plewnaik, F. Jeanmougin, D.G. Higgins, Nucleic Acid Res. **24**, 4876 (1997)
12. N. Saitou, M. Nei, Mol. Biol. Evol. **4**, 406 (1987)
13. M.L. Dyall-Smith, I. Lazdins, G.W. Tregear, I.H. Holmes, Proc. Natl. Acad. Sci USA. **83**, 3465 (1986)
14. C. Kirkwood, P.J. Masendycz, B.S. Coulson, Virology. **196**, 79 (1993)
15. L. Maunula, C.-H. von Bonsdorff, J. Gen. Virol. **79**, 321 (1998)

16. J. Blackhall, R. Bellinzoni, N. Mattion, M.K. Estes, J. La Torre, G. Magnusson, Virology. **189**, 833 (1992)
17. I. Lazdins, B.S. Coulson, C. Kirkwood, M. Dyall-Smith, P.J. Masendycz, S. Sonza, I.H. Holmes, Virology. **209**, 80 (1995)
18. A.Z. Kapikian, R.M. Chanock, in *Field's virology*, 3rd edn, ed. by B.N. Fildes, D.M. Knipe, P.M. Howley, R. B. Chanock, J. L. Melnick, T. P. Monath, B. Roizman, S.E. Straus (Lippincott-Raven, Philadelphia, 1996) pp. 1637–1708
19. Q. Jin, R.L. Ward, D.R. Knowlton, Y.B. Gabby, A.C. Linhares, R. Rappaport, P.A. Woods, R.I. Glass, J.R. Gentsch, Arch. Virol. **141**, 2057 (1996)
20. R.C. Bellinzoni, N.M. Mattion, D.O. Matson, J. Blackhall, J.L. La Torre, E.A. Scodeller, S. Urasawa, K. Taniguchi, M.K. Estes, J. Clin. Microbiol. **28**, 633 (1990)
21. M. Ciarlet, F. Liprandi, J. Cin. Microbiol. **32**, 269 (1994)
22. H. A. Hussein, A.V. Parwani, B.I. Rosen, A. Lucchelli, L.J. Saif, J. Clin. Microbiol. **31**, 2491 (1993)
23. N. Santos, R.C.C. Lima, C.M. Nozawa, R.E. Linhares, V. Gouvea, J. Clin. Microbiol. **37**, 2734 (1999)
24. T.A. Teodoroff, H. Tsunemitsu, K. Okamoto, K. Katsuda, M. Kohmoto, K. Kawashima, T. Nakagomi, O. Nakagomi, J. Clin. Mirobiol. **43**, 1377 (2005)