Nucleotide sequence comparison of the VP8* gene of rotaviruses possessing the AU-1 gene 4 allele

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Of the five currently recognized alleles of the human rotavirus VP4 gene, the AU-1 allele has captured attention because of its possible non-human origin. The 5' 750 nucleotide region of the VP4 gene, encoding the VP8* fragment [amino acids (aa) 1 to 241] and the connecting peptide (aa 242 to 247), from 13 human and two feline rotavirus strains possessing the AU-1 allele was highly conserved both at the nucleotide sequence (92.8 to 99.7% identity) and amino acid level (95.5 to 100% identity) irrespective of the year and the place of isolation or of the host species from which these viruses were isolated. This is consistent with the hypothesis that the AU-1 allele of the VP4 gene has been maintained in both human and feline rotavirus gene pools.

Group A rotavirus, a member of the family Reoviridae, is the major aetiological agent of acute diarrhoea in infants and young children worldwide (Kapikian & Chanock, 1990). The rotavirus genome consists of 11 dsRNA segments encased in a double-layered capsid (Estes & Cohen, 1989). The outer capsid spike protein VP4, which is encoded by gene segment 4 (Kalica et al., 1983), has been implicated in a number of biologically important functions such as viral neutralization (Hoshino et al., 1985; Offit & Blavit, 1986), viral virulence (Offit et al., 1986), haemagglutination (Kalica et al., 1983), restriction of the growth of certain rotavirus strains in cell culture (Greenberg et al., 1983) and in mice (Offit et al., 1986), and protease-enhanced plaque formation (Kalica et al., 1983). Hybridization and sequencing studies have shown the presence of at least 11 distinct VP4 gene alleles which are thought to define 11 distinct P (protease-sensitive protein) serotypes (Estes & Cohen, 1989; Hardy et al., 1992; Huang et al., 1992; Qian & Green, 1991).

Of 11 VP4 gene alleles, at least five alleles have been found in human rotaviruses; they are the Wa allele carried by symptomatic human rotaviruses possessing VP7 serotype G1, G3, G4 or G9, the DS-1 allele carried by symptomatic human rotaviruses possessing VP7 serotype G2, the M37 allele carried by asymptomatic nursery strains possessing VP7 serotype G1 to G4, the AU-1 allele carried by human rotavirus strains K8 (G1) and AU-1 (G3), and the 69M allele carried by human rotavirus strain 69M (G8). The AU-1 allele, which corresponds to VP4 serotype 3 (P serotype 3) according to the numbering system proposed by Gorziglia et al. (1990) or P serotype 9 according to the numbering system proposed by Estes & Cohen (1989), has been shown to be highly conserved between human rotavirus strain AU-1 and feline rotavirus strain FRV-1 (Isegawa et al., 1992a). This suggests that transmission of a feline rotavirus to a human has occurred (Nakagomi & Nakagomi, 1989). With an increasing number of rotavirus strains from symptomatic human infections in Japan (Gunasena et al., 1993; Nakagomi et al., 1989b; Nakagomi & Nakagomi, 1991) and in Italy (Gerna et al., 1990, 1992) being identified as possessing the AU-1 allele, an alternative possibility has been discussed, namely that the AU-1 allele has long been maintained in human rotaviruses, and the cat from which strain FRV-1 was isolated might have been infected with a human virus similar to strain AU-1. Although hybridization assays are a valuable means for examining the genetic relatedness of the whole constellation of the genome in this group of viruses, the precise degree of genetic homology among their VP4 genes has not been

The nucleotide sequence data reported in this paper are deposited in the DDBJ, EMBL and GenBank nucleotide sequence databases under the accession numbers D14613 (AU1115), D14615 (AU228), D14614 (AU125), D14616 (AU720), D14616 (AU379), D14617 (AU387), D14619 (AU785), D14620 (AU938), D14623 (PA151), D14624 (PCP5), D14622 (MZ58) and D14621 (Cat2).
Fig. 1. Predicted amino acids of the VP8* fragment of AU-1 VP4. The corresponding amino acid sequences of another 14 rotaviruses possessing the AU-1 allele are shown below that of strain AU-1 only where they differ.

determined. Such information is clearly important for a better understanding of the interrelationships of the AU-1 allele carried by rotavirus strains derived from diverse geographic and temporal origins and from different host species. Therefore, we have compared the nucleotide sequence of the 750 base 5' one-third of the VP4 gene of 13 human and two feline rotavirus strains carrying the AU-1 allele. The rationale for choosing this region for comparison was the fact that VP8* amino acids (aa) 1 to 241 and the connecting peptide (aa 242 to 247) have been shown to be most divergent among rotavirus strains (Estes & Cohen, 1989).


Rotaviruses were grown in MA104 cells in the presence of 0.5 μg of trypsin per ml. The infected cell culture
harvest was clarified by a low-speed centrifugation and the supernatant was pelleted by two cycles of ultracentrifugation, one at 36000 r.p.m. for 3 h in a Hitachi RP42 rotor and another through a 30% (w/v) sucrose chloride and 5 mM-EDTA. Single-shelled particles were recovered by caesium chloride density equilibrium ultracentrifugation.

Single-stranded RNAs (mRNAs) were prepared by in vitro transcription of the genomic dsRNAs with a virion-associated transcriptase as described previously (Nakagomi et al., 1989 a); mRNAs served as templates for cDNA synthesis of the region of gene segment 4 corresponding to VP8* by a combination of reverse transcription and PCR. Briefly, the mRNA was reverse-transcribed with a downstream consensus primer, 817/794 (CTCTATTATATTGCATTTCTTTCC) which is complementary to nucleotides (nt) 794 to 817 of the VP8* segment of the genomic dsRNA. The PCR products were directly sequenced by the cassette-ligation mediated PCR method previously described in detail (Isegawa et al., 1992 a).

We determined the nucleotide sequences of the VP8* fragment (the proteolytic product of the amino-terminal one-third of the VP4 protein) of 11 human rotavirus strains and one feline rotavirus strain possessing the AU-1 allele. The sequences were aligned with each other and with those of human strains AU-1 and K8 and feline strain FRV-1 previously sequenced (Taniguchi et al., 1989; Isegawa et al., 1992 a). Inspection of the aligned nucleotide and predicted amino acid sequences of the 13 human and two feline rotavirus strains possessing the AU-1 allele revealed that these genes were highly conserved irrespective of the year and the place of isolation or of the host species from which these viruses were isolated (Fig. 1). The AU-1 VP8* sequence was different from that of any one of the other 14 strains at 86 nucleotide positions. At 48 nucleotide positions, the substitution was shared by two or more strains. Most of the changes occurred, however, at the third position of a codon and did not alter the amino acid composition of the protein. For example, there were 35 substitutions between the sequences of strain AU-1 and strain MZ58 (the most divergent); of these 35 substitutions, 32 were at the third codon positions and only one of the 32 altered the amino acid sequence. Only five substitutions were present between the VP8* sequences of strain AU-1 and strain AU125 and none of them induced amino acid changes, resulting in 100% amino acid identity between the two strains.

It has been shown previously that the VP8* fragment of strains AU-1, FRV-1 and K8 is one amino acid longer than that found in other human rotavirus isolates but equal to that of animal rotaviruses due to an insertion of threonine after amino acid residue 135 (Isegawa et al., 1992 a; Taniguchi et al., 1989). This threonine residue was conserved in all of 12 additional strains sequenced in this study (Fig. 1). Since this threonine residue is conserved in most animal rotavirus VP8* except SA11 VP8* in which aspartic acid is present instead of threonine (Estes & Cohen, 1989), the VP8* segment of the AU-1 allele has a structure in common with animal rotavirus VP8*, suggesting an animal rotavirus origin for this allele.

Table 1 presents a matrix of pairwise sequence identities between each VP8* sequence at the nucleotide and amino acid levels. The VP8* sequences of the strains possessing the AU-1 allele were divergent from each other by 0-3 to 6-2% at the nucleotide level and by

Table 1. Percentage identity of nucleotide (above diagonal) and amino acid (below diagonal) sequence among rotavirus strains for the AU-1 allele in the region between nucleotides 1 and 750 of the VP4 gene (aa 1 to 247)
reported a high sequence conservation among members of a single VP4 gene allele by determining the VP4 sequences (although only for the VP8* fragment) of a reasonable number of natural isolates. Previously, only very limited numbers of the variation among members of a single VP4 gene allele by human rotavirus strains derived from asymptomatic nursery strains and VP7 gene studies have been shown to share VP4 antigenic specificity with isolates. This study has provided information on the sequence variation among members of a single VP4 gene allele by determining the VP4 sequences from some distinct VP4 gene alleles have been determined. For example, Gorziglia et al. (1988) reported a high sequence conservation among members of the VP4 gene alleles corresponding to VP4 serotype 1 subtype A (or P8) (92.2 to 97% nucleotide identity among three strains), among members of the VP4 gene alleles corresponding to VP4 serotype 1 subtype B (or P4) (98.6% nucleotide identity between two strains), and among those corresponding to VP4 serotype 2 (or P6) (95.5 to 97.5% nucleotide identity among four strains). The VP4 of porcine rotavirus strain Gottfried, which has been shown to share VP4 antigenic specificity with human rotavirus strains derived from asymptomatic neonatal infections (VP4 serotype 2 or P6) (Gorziglia et al., 1990; Kang et al., 1989), is closely related to the VP4s of neonatal human strains (87.1 to 88.1% amino acid identity) and Gottfried VP4 and the VP4s of neonatal human strains are considered to have diverged from a common ancestor (Gorziglia et al., 1990).

Sequence divergence among rotavirus strains of the same G serotype but originating from different host species has been documented. Nishikawa et al. (1989) determined the VP7 sequences of 27 human and animal rotavirus strains of serotype G3 in order to examine genetic variation within strains of identical G serotype. The VP7s of G3 viruses have an overall sequence identity of 85% or higher and a value of 95% or more was observed among G3 strains derived from the same animal species with the exception of two feline strains, Cat2 and Cat97 (90.8% amino acid identity) (Nishikawa et al., 1989). A high sequence conservation (93.8 to 99.7% nucleotide identity and 95 to 100% amino acid identity) in the VP8* region of the VP4s of the rotavirus strains possessing the AU-1 allele was observed irrespective of the origins of host species. The precedent of VP4 genes carried by porcine Gottfried virus and asymptomatic nursery strains and VP7 gene studies suggest that the AU-1 allele of the VP4 gene has been maintained in both the human and feline rotavirus gene pools, probably by frequent interspecies transmission.

Although the 15 strains examined in this study carried a highly conserved VP4 gene, their genetic composition was not necessarily similar. Most of the 11 genomic RNA segments of AU1115, AU228, AU125, AU720, AU379, AU387, AU783, AU938 and FRV-1 formed hybrids with the corresponding transcription probes prepared from the AU-1 strain (Nakagomi et al., 1989a, b; Nakagomi & Nakagomi, 1989; Nakagomi & Nakagomi, 1991; O. Nakagomi et al., unpublished), suggesting that their genetic similarity extended to most of the gene segments (i.e. members of the AU-1 genogroup). On the other hand, strains K8, PAI51, PCP5, MZ58 and Cat2 were shown by RNA–RNA hybridization studies to be naturally occurring intergenogroup reassortants (Gerna et al., 1992; Mochizuki et al., 1992; Nakagomi et al., 1992a, b). Further studies will, however, be required to determine whether the AU-1 allele initially originated in feline rotaviruses or in human rotaviruses.

Part of the nucleotide sequence analysis was done with a Vax computer at the Institute of Medical Science, University of Tokyo, and we thank Drs A. Ito and I. Saito for their generosity. This work was supported in part by a grant-in-aid for scientific research from the Ministry of Education, Science and Culture of Japan.

References


(Received 16 February 1992; Accepted 2 April 1993)