Incidence of Group C Human Rotavirus in Central Australia and Sequence Variation of the VP7 and VP4 Genes

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Human group C rotavirus was identified in central Australia in each of eight years over a 16-year period between 1982 and 1997. Cases occurred either sporadically but over a relatively short period of time or as clustered outbreaks. These are the only reports of human group C rotavirus in Australia other than that of a single case reported approximately 1,800 km away in 1982. The electrophoretic genome profiles of isolates were identical for all those identified within the same year but different between those identified in different years. The VP7 genes of four isolates identified in four different years over a 7-year period between 1987 and 1993, and the VP4 genes of two of these isolates showed relatively little variation in genome and deduced amino acid sequence upon comparison of the equivalent genes between isolates. The sequences were also very similar to those from the corresponding genes from most of the human group C rotavirus isolates from other countries. This continues the observation of a high degree of gene sequence conservation among human group C rotaviruses worldwide.

Rotaviruses are major causes of acute diarrhea in humans and animals throughout the world, and on the basis of antigenic cross-reactivity and genomic properties they are classified into seven serogroups A to G (17). All seven groups have been found to be associated with infection in animals but only three (A to C) are associated with diarrhea in humans (17). Group A rotaviruses are the most common cause of severe diarrhea in infants and young children, but they also infect adults (10). Group C rotavirus infection in humans occurs both in children and adults, usually in sporadic cases or clustered outbreaks, and has now also been identified worldwide (1, 3, 8, 9, 11, 12, 14–17, 20). However, compared to group A rotaviruses, the identification of group C rotaviruses has been far less common, and neither the overall disease burden nor the epidemiology of this group of rotaviruses is yet entirely clear (9).

In relation to this, identification of group C rotaviruses would have been hindered since commercially used enzyme immunoassays (EIAs) would generally not recognize the group C rotavirus-specific antigen on the inner capsid protein VP6, which carries the group-specific antigen of a rotavirus group. Polyacrylamide gel electrophoresis (PAGE), which can be used to distinguish between different serogroups of rotavirus (17), and electron microscopy are not often used to detect rotavirus in stool specimens (9). Confirmatory tests, such as with reverse transcription-PCR (RT-PCR) with group C rotavirus-specific primers (5, 8) and EIAs with group C rotavirus-specific reagents have not been widely used, usually only in reference laboratories (9).

The structure of group C rotaviruses is similar to that of group A rotaviruses with virus particles characterized by dou-

ble capsids enclosing a genome of 11 double-stranded RNA segments (17). The genome segments, however, form basic groupings on polyacrylamide gels different from those of group A and the other serogroups of rotaviruses (17). The coding assignments of group C rotavirus genes also appear to differ from those of group A rotaviruses for several major structural proteins, including VP4, VP6, and VP7 (9).

A high degree of variability in the sequence of the gene coding for VP7, the major outer capsid protein of rotaviruses, is evident among group A rotaviruses, both among and between human and animal strains. Sequence variation is also evident between VP7 genes of human and animal group C rotaviruses, but among human group C rotavirus isolates themselves from many different parts of the world remarkable sequence conservation has been noted in this gene (1, 3, 6, 9, 11). A high degree of conservation has also been evident between the sequences of the VP4 genes of human group C rotavirus isolates from different parts of the world, but a greater difference was noted between these sequences and those of the VP4 genes of bovine and porcine group C rotavirus strains (1, 4).

Human group C rotaviruses have generally proved difficult to grow in cell culture, and this has hindered the use of neutralization assays to serotype them. Type-specific monoclonal antisera, such as those available for group A rotaviruses, are not available for human group C rotaviruses. Relatedness between human group C rotaviruses has therefore thus far only been inferred from sequence analysis of (essentially) the VP7 genes (9).

We report here on the incidence of human group C rotavirus over 16 years in central Australia and on the sequences of the VP7 genes of four and the sequences of the VP4 genes of two of the isolates identified during a 7-year span within this period.

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TABLE 1. Number of cases of group C and group A rotavirus detected in the years in which group C rotavirus was detected

Rotavirus group	No. of cases in:								
	1982	1984	1985	1987	1988	1990	1993	1995	1997
C	1	1	2	8	3	4	5	2	2
A	68	45	64	62	32	63	92	77	49

MATERIALS AND METHODS

Stool samples. For determination of the incidence of human group C rotavirus in central Australia, stool samples from infants and young children admitted to Alice Springs Hospital with gastroenteritis were examined. Alice Springs Hospital serves an area of central Australia within an approximately 500-km radius of Alice Springs and a total population of approximately 25,000. The area, with the exception of Alice Springs itself, is very sparsely populated, with widely dispersed small settlements scattered throughout.

Virus detection. Rotavirus was detected in clarified stool samples by electron microscopy, and then group C rotavirus was specifically identified by comparison of the virus genome profiles obtained on polyacrylamide gels with genome profiles of group C rotaviruses isolated elsewhere (17). Genome profiles were visualized by silver staining after PAGE of extracted viral RNA. For confirmation as group C rotaviruses, if sequencing of one or more genes of an isolate was not carried out, the PCR method of Gouvea et al. (5) was used.

Extraction of viral RNA for PCR and sequencing. Homogenized 10 to 20% stool suspensions in phosphate-buffered saline (pH 7.2) were clarified by centrifugation at $13,000 \times g$ for 10 s, and the supernatant adjusted to 1% with respect to sodium dodecyl sulfate and 0.1 M with respect to sodium acetate buffer (pH 5.4). After this was mixed, an equal volume of 1:1 phenol-chloroform was added, the mixture vortex mixed for 30 s, and the phases were separated by centrifugation at $13,000 \times g$ for 30 s. Rotavirus RNA was extracted from the aqueous phase by using a commercial glass powder preparation (RNAID kit; Bio 101, Vista, Calif.). After the glass powder matrix had been resuspended in the aqueous phase by vortexing, the mixture was rocked for several minutes before the matrix was pelleted, and the supernatant was removed. The matrix, with viral RNA attached, was then washed twice with the supplied wash buffer by resuspension in 500 μ l of the buffer and then pelleted again. After the second wash the matrix-RNA was resuspended in 25 μ l of sterile distilled water, and this mixture was held at 55°C for 10 min in a water bath. The matrix was then pelleted, and the supernatant containing the viral RNA was stored at -80°C until required.

RT-PCR. For RT and transcription of the full-length of the VP7 gene, the primers used comprised the common first 21 bases of this gene in the forward direction for group C rotaviruses and the common first 21 bases of the complementary strand in the reverse direction. For RT and transcription of the VP4 gene, the primers comprised the common first 20 bases of the gene in the forward

direction and the common first 20 bases of the complementary strand in the reverse direction. For the reaction, after a denaturing step at 97°C for 5 min, followed immediately by chilling in an ice-salt bath, 5 μ l of extracted RNA plus 3.5 μ l of dimethyl sulfoxide was added to a 41- μ l mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 4 mM MgCl₂, 1 mM concentrations (each) of dATP, dCTP, dGTP and dTTP, 500 ng of each primer, and 12.5 U of avian myeloblastosis virus reverse transcriptase (Roche). After RT at 42°C for 60 min, 2.5 U of *Taq* DNA polymerase was added, and amplification was carried out for 30 cycles of 94°C for 1 min, 42°C for 2 min, and 72°C for 2 min.

Nucleotide sequencing. Primers of 20 bases derived from the published sequences of the VP7 gene of the human group C rotavirus Bristol strain (6) and the VP4 gene from the same strain (4), sited at approximately 200-base intervals along both strands of the genes, were used in further PCR amplification of subsections of these genes for sequencing. The primers used for RT or amplification were also utilized for this. The reaction mixture and PCR cycling conditions used were as outlined above except that 2 mM MgCl₂ and 200 ng of each of the primers was used. The PCR products were then excised after having been electrophoresed on 1.8% agarose gels and further purified by using the Qiaex II DNA purification kit (Qiagen) according to the manufacturer's instructions. Manual sequencing was carried out by using the Thermo-Sequenase radiolabeled terminator cycle sequencing kit (Amersham Pharmacia Biotech) after a pretreatment of the purified cDNA samples with the enzymes from the Amersham Pharmacia Biotech PCR product presequencing reagent kit according to the manufacturer's instructions. Sequences were generated in both directions, and a consensus sequence was obtained.

The CLUSTAL W multiple sequence alignment program was downloaded from the European Bioinformatics Institute website (<http://www.ebi.ac.uk/clustaw>) and used to generate the phylogenetic trees. The method used was the neighbor-joining method. Since no difference in the trees drawn with correction (by the Kimura method) or without correction for multiple substitutions was observed, the trees shown are without such correction.

Nucleotide sequence accession numbers. The VP7 gene sequences of isolates CHRV/A90L and CHRV/A93M have been assigned accession numbers AY392446 and AY392447, respectively, by GenBank, and the VP4 gene sequences of isolates CHRV/A87J and CHRV/A93M have been assigned accession numbers AY395069 and AY395070, respectively. The VP7 gene sequence of isolate CHRV/A87J is identical to that of isolate ASP/87 (GenBank accession number U20990), and that of isolate CHRV/A88G is identical to that of isolate ASP/88 (GenBank accession number U20991).

RESULTS

Epidemiology. Group C rotavirus was identified in stool samples from gastroenteritis patients in central Australia in 1982 (one case), 1984 (one case), 1985 (two cases), 1987 (eight cases), 1988 (three cases), 1990 (four cases), 1993 (five cases),

TABLE 2. Sequence comparison of the VP7 gene of human group C rotaviruses^a

Isolate	Sequence comparison (%) of isolate (origin):													
	CHRV/A87J	CHRV/A88G	CHRV/A90L	CHRV/A93M	Preston (United Kingdom)	Belém (Brazil)	Solano (Argentina)	RI-1/ad957 (United States)	OK450 (Japan)	K9304 (Japan)	208 (China)	KA4/949 (Sweden)	Jajeri (Nigeria)	Moduganari (Nigeria)
A87J		98.9	99.0	98.6	98.3	98.2	98.3	99.1	95.9	98.5	98.5	98.2	96.3	97.2
A88G	99.4		99.6	98.1	98.1	98.0	97.8	99.1	95.9	99.5	98.1	97.9	96.6	96.9
A90L	99.4	100		97.9	98.1	97.8	97.6	98.9	95.9	99.5	97.9	97.7	96.6	96.9
A93M	98.4	97.9	97.9		97.5	97.6	98.4	98.4	95.0	97.8	99.3	98.3	96.0	96.5
Preston	99.4	99.4	99.4	98.2		97.8	97.3	98.9	95.4	97.7	97.6	97.4	96.3	96.6
Belém	99.1	99.1	99.1	97.6	99.1		97.4	98.8	95.3	97.7	97.6	97.6	96.1	96.8
Solano	97.6	97.9	97.9	96.7	97.6	97.3		98.4	95.0	97.5	98.6	98.8	95.7	96.4
RI-1/ad957	99.7	99.7	99.7	98.2	99.7	99.4	98.2		96.3	98.8	98.5	98.3	97.3	97.7
OK450	96.7	96.7	96.7	95.2	96.7	96.4	95.5	97.0		95.6	94.9	94.9	94.4	94.4
K9304	98.8	99.7	99.7	98.2	98.8	98.5	97.0	99.1	96.7		97.8	97.5	96.4	97.0
208	98.5	98.5	98.5	98.8	98.4	98.2	97.9	98.8	96.1	97.9		98.3	95.7	96.3
KA4/949	99.1	99.1	99.1	98.2	99.1	98.8	98.2	99.4	96.4	98.5	98.8		95.7	96.4
Jajeri	98.5	98.5	98.5	97.0	98.5	98.2	96.7	98.8	95.8	97.9	97.6	98.2		97.6
Moduganari	98.5	98.5	98.5	97.0	98.5	98.2	96.7	98.8	95.8	97.9	97.6	98.2	99.4	

^a Numbers at the top right indicate results of pairwise comparison of the nucleotide sequences, and numbers at the bottom left indicate results of pairwise comparison of the deduced amino acid sequences. For the non-Australian isolates the GenBank accession numbers are as follows: Preston, X77258; Belém, X77256; Solano, AF120478; ad957, U20993; OK450, D87544; K9304, AB004250; 208, AB008671; KA4/949, AF225561; Jajeri, AF323982; and Moduganari, AF323979.

FIG. 1. Alignment of the deduced VP7 amino acid sequences of the 4 Australian and 10 non-Australian human group C rotavirus isolates listed in Table 2. The sequences are numbered from the first methionine residue at the start codon, i.e., positions 49 to 51 of the VP7 gene. Identical amino acids are represented by a dot.

1995 (two cases), and 1997 (two cases). Table 1 gives a comparison of the numbers of group C and group A rotavirus cases detected in each of the years in which group C rotavirus was detected. The ages of the group C rotavirus patients ranged from 2.5 months to 2.5 years. In each year all of the group C rotavirus cases occurred within a relatively short period of time, except in 1987 when they occurred over approximately a month and a definable severe outbreak of gastroenteritis could be attributed to group C rotavirus. In this outbreak not only were the symptoms more severe than usual for rotavirus gastroenteritis in the area, but it occurred in a hot summer month rather than in the colder months, as is generally the case for

rotavirus in the region. From the symptoms in other gastroenteritis patients at the time it is also highly likely that there were more than the eight positively identified cases.

Virus genome profiles. The electrophoretic genome profiles of all 28 of the central Australian isolates were characteristic of those of group C rotaviruses, all showing the typical 4-3-2-2 migration pattern. Profiles of isolates from within the same year were always found to be identical, but they were different between isolates from each of the eight years in which group C rotavirus was identified.

VP7 genes. The VP7 genes of isolates from 4 of the 8 years were able to be sequenced, these included CHRV/A87J (plus

FIG. 2. Phylogenetic tree of the VP7 genes of the 4 Australian (CHRV/A87J, CHRV/A88G, CHRV/A90L, CHRV/A93 M) and the 10 non-Australian (Preston, Belém, Solano, ad957, OK450, K9304, 208, KA4/949, Jajeri, and Moduganari) group C rotavirus isolates compared in Table 2.

FIG. 3. Phylogenetic tree of the amino acid sequences deduced from the VP7 genes of the 4 Australian (CHRV/A87J, CHRV/A88G, CHRV/A90L, and CHRV/A93 M) and 10 non-Australian (Preston, Belém, Solano, ad957, OK450, K9304, 208, KA4/949, Jajeri, and Moduganari) group C rotavirus isolates compared in Table 2.

two others) from 1987, CHRV/A88G (plus one other) from 1988, CHRV/A90L from 1990, and CHRV/A93 M from 1993. A comparison of the VP7 gene base sequences, as well as the deduced amino acid sequences between the central Australian isolates themselves, and the central Australian isolates and a number of human group C rotavirus isolates from other countries is shown in Table 2. A multiple alignment of the deduced VP7 amino acid sequences of the Australian and non-Australian isolates is also shown in Fig. 1. For the comparison, at least one isolate was selected from most of the countries in which human group C rotavirus had been identified and the VP7 gene sequence published.

Overall, identity between the central Australian isolates was high, as was that between the central Australian isolates and the 10 from eight other countries. The base and deduced amino acid sequences of the three different 1987 isolates tested were identical, as were those of the two different 1988 isolates tested. The lowest level of identity between central Australian isolates with respect to amino acid sequences was 97.9% (a

difference of 7 amino acids out of a total of 332) between the 1993 isolate and those from 1988 and 1990. The biggest difference between central Australian isolates and those from the other countries at the amino acid level was at 95.2% identity (a 16-amino-acid difference) between the central Australian 1993 isolate (CHRV/A93 M) and isolate OK450 from Japan. This was also the biggest difference at the amino acid level between any of the 14 isolates whose VP7 gene sequences were included in Table 2. Amino acid substitutions were generally variable (Fig. 1). The biggest difference with respect to base sequences and considering all isolates included in Table 2 was at 94.4% identity (a difference of 60 or 61 bases out of a total of 1063) between the Japanese isolate OK450 and the Nigerian isolates Moduganari and Jajeri. Overall, it was evident that the vast majority of differences in base sequence among all of the central Australian and overseas isolates were not reflected in differences in amino acid sequence.

It was notable that isolate OK450 was consistently the one

TABLE 3. Sequence comparison of the VP4 gene of human group C rotaviruses^a

Isolate	Sequence comparison (%) of isolate (origin):						
	CHRV/A87J	CHRV/A93M	Bristol (United Kingdom)	Belém (Brazil)	208 (China)	Jajeri (Nigeria)	Moduganari (Nigeria)
CHCRV/A87J		98.0	98.2	97.3	97.6	97.1	96.9
CHCRV/A93M	98.5		97.8	97.0	97.7	97.2	97.2
Bristol	98.5	98.1		98.0	97.2	96.9	97.0
Belém	98.0	97.6	99.2		96.7	96.1	96.2
208	98.5	98.1	97.7	98.0		98.0	97.8
Jajeri	97.8	97.4	97.3	97.3	98.8		99.6
Moduganari	97.7	97.3	97.2	97.2	98.4	99.6	

^a Numbers at the top right indicate results of pairwise comparison of the nucleotide sequences, and numbers at the bottom left indicate results of pairwise comparison of the deduced amino acid sequences. For the non-Australian isolates the GenBank accession numbers are as follows: Bristol, X79442; Belém, X79441; 208, AB008670; Jajeri, AF323981; and Moduganari, AF323980.

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MASSLYUQLISQNYISLNEILSDQQTNKVISDYVDAGNYTYAQLPPTTWGSGSILKSASFSTPEITGPHTNTVIEWSNLINTNTWLLYQKPLNSVRLKH 100
.....V.E.....E.....
.....V.....
.....V.....
.....
.....

GPDTYNSNLAAPFELWYKSGTTITSVYYNTINNQNKTHEANSCLILFWNEGSTQLEKQVVTFNWNVGGILIKPINSSRMKICMSGMENFNDSFNWENW 200
.....A.....D.....R.....
.....A.....T.....D.....R.....
.....A.....T.....D.....R.....
.....A.....T.....V.D.....N..R.....
.....A.....T.....V.D.....N..R.....

NHEFPRSNPGININMYTEYFLASSDPYTYLKNLQOPTVKTKIDMKMMKMNNDNSKLGDPINVSNIISKDSLWQEVQYVRDITLQCKILSEIVKGGGWYD 300
.....A.V.....
.....A.V.....
.....S.....
.....N..I.....I.....
.....N.....N.....I.....

YTSVTFKTVNHTYSYTRAGENVAHVITISFNNVKERAYGGSPLTFDKIGRFDILDTSYVYIDYDDSEIFKNMVYVRDVRADIGGFQYSYSSEMSYFQ 400
.....
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.....
.....
.....

IPVGSYPGLHSSRLQLVYDRCLLSQFTDYAALNSLRFIFRVVSTSGWFITTDINTRRVSAGTGFAYSDDGHVANTVGTISFISLIPSNPNYQTPASS 500
.....K.....V.....
.....
.....K.....
.....K.....
.....K.....S.....

TVRMDLERKINDLRDDFNALASSVALSDILSLAMSPLTFNLLSVPAITSSVKDVAASVMKKFRSTKMPKKAQNYREFIIGDLEEDVTNVARNNNSL 600
.....V.....
.....V.....
.....G.....V.....
.....G.....N.....V.....I.....
.....N.....V.....
.....N.....V.....

NYSIDTSAMMVSTTNRLQITDVFSEIVSRSDNFISNRSYRMIENTVHEITPTRSFSYDIKTLQQRNFDVDFYKLASQSPVVSIAIVDFATIKAIRD 700
.....V.....I.....I.....
.....R.....I.....S.....I.....
.....I.....R.....D..I.....S.....
.....I.....I.....
.....I.....
.....I.....

TYGISDDIYKLVASDAPTILSFNQNNPLIRNRTNLINQCKL 744
.....N.....S.....
.....
.....N.....D.....
.....N.....D.....S.....
.....N.....D.....S.....

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CHRV/A87J
 CHRV/A93M
 Bristol
 Belém
 208
 Jajeri
 Moduganari

FIG. 4. Alignment of the deduced VP4 amino acid sequences of the two Australian and five non-Australian human group C rotavirus isolates listed in Table 3. The sequences are numbered from the first methionine residue at the start codon, i.e., positions 21 to 23 of the VP4 gene. Identical amino acids are represented by a dot.

with the greatest differences to the other isolates. This was further illustrated in the phylogenetic trees of the VP7 genes and the deduced VP7 amino acid sequences of the 14 group C rotavirus isolates, as shown in Fig. 2 and 3. It was particularly evident in the case of the tree generated from the amino acid sequences. It was also evident that the four Australian isolates did not necessarily group together, isolate CHRV/A93 M, for example, grouped together with the Chinese isolate 208 in both the base and amino acid sequence derived trees.

VP4 genes. The VP4 genes of two of the central Australian group C rotavirus isolates were sequenced, those of CHRV/A87J from 1987 and CHRV/A93M from 1993, and comparison of sequences also revealed a high level of identity, 98.5% (a difference of 11 amino acids out of a total of 744) with respect

to amino acid sequences and 98.0% (a difference of 45 bases out of a total of 2283) with respect to base sequences (Table 3). A high level of identity was also evident when the sequences of the VP4 genes and deduced amino acid sequences of the central Australian isolates and those of the five overseas isolates for which sequences of this gene have been published were compared (Table 3). A multiple alignment of the deduced VP4 amino acid sequences of the Australian and non-Australian isolates is shown in Fig. 4. With respect to amino acid sequences, the biggest differences were between isolates Bristol (United Kingdom) and Belém (Venezuela) and the Nigerian isolate Moduganari with 97.2% identity (a difference of 21 amino acids). Amino acid substitutions were slightly less variable than for VP7 (Fig. 4). With respect to base sequences the

FIG. 5. Phylogenetic tree of the VP4 genes of the two Australian (CHRV/A87J and CHRV/A93) and five non-Australian (Bristol, Belém, 208, Jajeri, and Moduganari) group C rotavirus isolates compared in Table 3.

biggest difference was between the Belém and Jajeri (Nigeria) isolates at 96.1% identity (a difference of 89 bases). As in the case of the VP7 genes the vast majority of base changes between the various isolates were silent with respect to amino acid coding.

The relationship between the seven isolates with respect to VP4 is further shown in the phylogenetic trees of the VP4 genes and the deduced VP4 amino acid sequences shown in Fig. 5 and 6. In the case of VP4 the two Australian isolates did group together with respect to amino acid sequences.

DISCUSSION

Group C rotavirus was detected periodically but continuously over a 16-year period, between 1982 and 1997, in central Australia, generally as clustered cases with respect to temporal incidence. The only other report of group C rotavirus in Australia was in 1982 of a single case in Melbourne (16), approximately 1,800 km from central Australia. Therefore, despite relatively intensive surveys of rotavirus incidence throughout Australia, particularly in more recent years, it would appear that central Australia is the only region in Australia in which group C rotavirus is generally present. In the region, however, this is usually with the concurrent, continuous, and far more significant presence of group A rotaviruses.

With the widespread use of commercially available detection kits, which do not detect group C rotavirus, forming the basis of many rotavirus surveys it could, however, still be that individual cases of group C rotavirus infection in Australia (and elsewhere) have not been and are not being detected. Also, group C rotavirus is often not actively looked for, even though sensitive but also time-consuming and relatively expensive methods for detection such as EIA and RT-PCR are available. In the present study the use of the relatively insensitive method of electron microscopy, together with PAGE, to detect virus very probably meant that cases of group C rotavirus infection would have been missed.

In other countries serological surveys have suggested that group C rotavirus infections are far more common than actual virus identification may have indicated (2, 7, 13, 19). These surveys, however, also included adults, among whom there was usually a higher level of group C rotavirus seropositivity than

among infants and young children. Adults were not included in the survey reported on here and in central Australia adults are unlikely to visit a hospital for treatment for gastroenteritis unless it is very serious.

The present study is the first that has looked at the incidence of group C rotavirus over such a relatively long time period in the same region, making it a potentially useful one for following the evolution of the viruses. Central Australia is a very isolated region, although it is also a major national and international tourist destination. It is therefore unfortunate that the VP7 and VP4 genes of isolates spanning only 7 years were able to be sequenced.

Changes in the sequences of both the VP7 and VP4 genes were evident over the 7-year period but they were few, particularly relative to the differences in sequence between the equivalent human and animal group C rotavirus genes (1, 3, 4, 6, 9). This indicates that the evolution or drift of these genes is slow. It is also very much in keeping with the results of other studies in which the sequences of these human group C rotavirus genes from isolates from a number of different countries were compared and a very high degree of sequence conservation was found (1, 3, 4, 6, 9). Our results, including the VP7 and VP4 gene sequence comparisons between the central Australian and overseas isolates, therefore confirm and extend the findings presented in the other studies. These results also support the contention that human group C rotaviruses evolved relatively recently and possibly belong to a single globally distributed genotype (3, 6, 9).

Prior to the present study the VP4 gene sequences of only five different human group C rotavirus isolates had been published, and the present study has added the sequences of a further two, with the results continuing to show the very high degree of sequence conservation evident so far in this gene among isolates worldwide. Considering this relatively low number of isolates from which the VP4 genes have been sequenced, more human group C rotavirus isolates should be analyzed to confirm whether the level of conservation in this gene is really as high as currently appears.

It has become evident that as more human group C rotavirus isolates have been identified in different countries, a few have now been determined to have greater VP7 gene sequence

FIG. 6. Phylogenetic tree of the amino acid sequences deduced from the VP4 genes of the two Australian (CHRV/A87J and CHRV/A93 M) and five non-Australian (Bristol, Belém, 208, Jajeri, and Moduganari) group C rotavirus isolates compared in Table 3.

differences from most of the other such isolates identified than was apparent in earlier years. Certainly with differences greater than those published in some of the earlier VP7 gene sequence comparisons. Examples of such isolates are OK450 from Japan and to a lesser extent Solano from Argentina. It is the difficulty in adapting human group C rotavirus to cell culture that has hindered the use of neutralization for testing for actual antigenic differences between isolates. However, now that such adaptation has been achieved (18), it would be of interest to try to adapt particularly the more different isolates to growth in cultured cells and then to test whether the greater differences in amino acid sequence were manifested as significant antigenic and possibly serotypic differences.

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