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The Correct Sequence of the Porcine Group C/Cowden Rotavirus Major Inner Capsid Protein Shows Close Homology with Human Isolates from Brazil and the U.K.

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Amino acid sequence alignments between the human group C/Bristol and the published porcine group C/Cowden VP6 proteins have revealed a region of extreme sequence divergence. We have been unable to confirm the nucleotide sequence of the Cowden VP6 gene corresponding to this region of divergence. Direct sequencing of a PCR-amplified cDNA pool has revealed a frame shift, and three nucleotide changes, within the published sequence of the porcine (Cowden) VP6 gene. The corrected sequence of the porcine protein revealed a closer homology with VP6 from the Bristol strain and two new human group C rotavirus isolates. Atypical rotaviruses have been detected in the feces of children living in Belém, Brazil, and Preston, U.K. Direct sequencing of PCR-amplified cDNA corresponding to the VP6 gene of one isolate from each location confirmed the presence of a group C rotavirus. The complete nucleotide sequences of the VP6 genes from the group C/Belém and C/Preston rotaviruses contained an open reading frame of 1185 nucleotides (395 amino acids; deduced M_r 44,669 Da). The Belém VP6 gene demonstrated 97.9% nucleotide homology with the human group C/Bristol VP6 gene and 83.4% nucleotide homology (91.6% deduced amino acid homology) with the corrected porcine group C/Cowden sequence. The Preston VP6 gene demonstrated 99.6% nucleotide homology with the human group C/Bristol VP6 gene and 84.0% nucleotide homology (91.6% deduced amino acid homology) with the corrected porcine group C/Cowden sequence. Remarkably, the deduced amino acid sequence of the Brazilian strain was identical to that of the U.K. isolates.

Rotaviruses are the single most important viral agent of infantile gastroenteritis and are responsible for a high degree of morbidity and mortality within developing countries (*1–3*). Infective rotavirus particles possess a double-shelled capsid layer that surrounds a segmented, double-stranded RNA (dsRNA) genome. Each monocistronic genome segment encodes either structural capsid proteins or non-structural proteins essential for viral replication (*4*).

At present rotaviruses are classified into seven groups (A–G) (*5, 6*) based on the immunological structure of the major inner capsid protein, VP6, estimated to constitute 51% of the total virion protein (*7*). Current diagnostic methods for rotavirus rely upon the immunological detection of this protein from clinical samples by an enzyme-linked immunosorbent assay (ELISA). However the ELISA can detect only group A rotavirus infections. There are no antigen-based detection systems currently available for the non-group A rotaviruses.

Only three groups of rotavirus (A–C) (*6*) are known to infect humans, of which group A rotaviruses are the most common cause of diarrheal disease in children under 5 years of age. All members within each rotavirus group exhibit common antigenic features of VP6, the group antigen, and genomic RNA cross-hybridization (*5, 8*). Human non-group A rotavirus isolates have been identified and initially assigned to a group according to the migration pattern of the 11 dsRNA segments (electropherotyping) and terminal fingerprinting of the genome segments (*8*).

Group C rotaviruses were first isolated and identified from the feces of pigs with severe gastroenteritis (*9, 10*). Subsequently, numerous outbreaks of gastroenteritis in humans have been unequivocally attributed to the group C rotavirus (*11, 12*). One case resulted in the death of a small child in Bristol, U.K. (*13*). Because of the paucity of material available in clinical samples only three isolates of the group C rotavirus have been further characterized at the genomic level (*14–16*). The nucleotide sequences of the gene encoding the common group antigen (VP6) of two of these isolates (a porcine and a human group C rotavirus) (*14, 15*) have recently been reported, and we have included important corrections to the published sequence from the porcine group C/Cowden rotavirus. Interestingly segment 5 of the group C genome has been identified as coding for VP6 (*15*), which contrasts with segment 6 of the group A rotaviruses.

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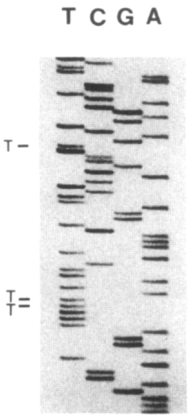
A



B

Published sequence

Nucleotide sequence	Amino acid sequence	
ATCAAGACGTGAGAATCCTGTATATGAGTATAAAAATCC	NWNAQSRRENPVYEYKNPMLFEYKNSYI	group C/Belém
.....	.....	group C/Bristol
G...-.G.....A..T.....A...-.....	..S...GVRIQ-FMNI...V...R....	group C/Cowden

Corrected Cowden sequence

Nucleotide sequence	Amino acid sequence	
ATCAAGACGTGAGAATCCTGTATATGAGTATAAAAATCC	NWNAQSRRENPVYEYKNPMLFEYKNSYI	group C/Belém
.....	.....	group C/Bristol
G.....G.....A..T.....A.....	..S.....V...R....	group C/Cowden

FIG. 1. Corrected nucleotide sequence of the porcine group C/Cowden rotavirus major inner capsid protein gene. (A) Autoradiograph showing the porcine group C/Cowden VP6 sequence of the noncoding DNA strand within the highlighted region of divergence. The arrows indicate the extra base additions to the published sequence. T, C, G, and A represent the appropriate dideoxynucleotide chain terminations. (B) Comparison of the nucleotide and deduced amino acid sequences of the major inner capsid protein gene of the Cowden strain and two of the human isolates. The top panel shows a comparison with the published Cowden sequence data, and the bottom panel shows a comparison of the corrected Cowden sequence data.

The presence of atypical rotaviruses has been reported in the feces from children with diarrhea living in Belém, Pará, Brazil (*17, 18*), and Preston, U.K. (Dr. E. O. Caul, personal communication). The presence of rotavirus particles was confirmed by electron microscopy, but ELISA tests for the rotaviral group A antigen proved negative. Genome electropherotyping tentatively classified the viral agents as a group C rotavirus. We also report the characterization of the VP6 gene from the Brazilian and a new U.K. isolate, and confirm the classification as a group C rotavirus based on the extensive nucleotide and protein sequence homology with the equivalent gene from the human group C/Bristol isolate.

The porcine group C/Cowden, the human group C/Bristol, and the human group C/Belém rotavirus fecal samples used in this study have been reported previously (*13, 17, 21*). The human group C/Preston rotavirus isolate was kindly provided by Dr. E. O. Caul, Bristol, U.K. Genomic dsRNA was extracted from RNase-T1-treated samples using RNAzol B (Biogenesis, Ltd.) and purified using Geneclean II (Stratech Scientific) as described (*19*).

VP6-specific cDNA was synthesized using SuperScript reverse transcriptase (Life Technologies) and amplified by the polymerase chain reaction (PCR) from genomic dsRNA. The oligonucleotide primers used for the cDNA synthesis and PCR amplification were synthesized on a model 381A automated synthesizer (Applied Biosystems, Inc.) using β-cyanoethyl phosphoramidite (CEP) chemistry. The VP6 gene of the human group C/Bristol rotavirus was cloned as described (*19*), which allowed determination of the terminal nucleotide sequences. The primers used for the two human isolates corresponded to the 5′ terminus (5′-GGCTTTAAAAATCTCATTCA-3′) and the 3′ terminus (5′-AGCCACATAGTTCACATTTC-3′) of the Bristol VP6 gene. The primers used for analysis of the porcine VP6 gene corresponded to the 5′ terminus (5′-GGCATTTAAAATCTCATTCAC-3′) and the 3′ terminus (5′-AGCCACATAGTTCACATTTC-3′) of the group C/Cowden VP6 gene (*14*). Each primer was synthesized with and without a 5′ terminal biotin group. Biotinylated primers were synthesized using a terminal 5′-biotinyl-C_{16}-CEP (Peninsula Laboratories Europe, Ltd.).

cDNA was synthesized in a 50-μl reaction containing denatured rotaviral dsRNA, 50 m*M* Tris–HCl, pH 8.3, 75 m*M* KCl, 3 m*M* $MgCl_2$, 10 m*M* DTT, 7% (v/v) DMSO, 100 μg/ml BSA, 100 ng of each nonbiotinylated primer, 40 U RNAsin (Promega), 500 U SuperScript (Life Technologies) reverse transcriptase and incubated at 42° for 1 hr. After treatment with 3 U RNase H (Promega) at 37° for 20 min the cDNA was purified by Sephacryl S-400 spin column chromatography (*22*).

The purified cDNA products were used as a template for the PCR amplification using recombinant *Taq* DNA polymerase (Promega) with one biotinylated and one nonbiotinylated primer. The primers used were the same as for the cDNA synthesis. PCR amplification was performed in a GeneAmp 9600 (Perkin–Elmer Cetus) and comprised 35 cycles of denaturation at 94° for 15 sec, primer annealing at 60° for 20 sec and DNA polymerization at 72° for 30 sec. The final cycle was followed by an additional polymerization step at 72° for 3 min.

Biotinylated PCR material was purified from excess biotinylated primers using Geneclean II (Stratech Scientific), adsorbed to Dynabeads M-280 Streptavidin (20 μl PCR material per 400 μg Dynabeads), and processed for DNA sequencing as described by the manufac-

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        10        20          30          40          50          60          70          80
GCATTTAAAATCTCATTCACA ATG GAT GTG CTT TTT TCC ATT GCG AAA ACT GTT TCA GAT CTC AAA AAG AAA GTT GTA GTT
                      Met Asp Val Leu Phe Ser Ile Ala Lys Thr Val Ser Asp Leu Lys Lys Lys Val Val Val      20

       90          100         110         120         130         140         150
GGT ACA ATT TAT ACC AAT GTG GAA GAT ATC ATT CAA CAG ACA AAT GAA TTG ATT AGA ACC TTA AAT GGC AAT ACA TTT
Gly Thr Ile Tyr Thr Asn Val Glu Asp Ile Ile Gln Gln Thr Asn Glu Leu Ile Arg Thr Leu Asn Gly Asn Thr Phe      46

         170         180         190         200         210         220         230
CAT ACT GGT GGA ATT GGA ACA CAG CCT CAA AAA GAG TGG AAC TTT CAA CTT CCT CAA CTG GGT ACA ACT CTG TTA AAT
His Thr Gly Gly Ile Gly Thr Gln Pro Gln Lys Glu Trp Asn Phe Gln Leu Pro Gln Leu Gly Thr Thr Leu Leu Asn      72

240         250         260         270         280         290         300         310
TTA GAT GAT AAT TAT GTC CAG GCA ACT AGA AGT GTG ATT GAT TAT TTA GCA TCA TTT ATA GAA GCT GTA TGT GAT GAT
Leu Asp Asp Asn Tyr Val Gln Ala Thr Arg Ser Val Ile Asp Tyr Leu Ala Ser Phe Ile Glu Ala Val Cys Asp Asp      98

   320         330         340         350         360         370         380         390
GAA ATA GTT AGA GAG GCT TCA AGA AAT GGA ATG CAA CCT CAG TCT CCA ACT CTT ATA GCA CTT GCA TCA TCA AAA TTT
Glu Ile Val Arg Glu Ala Ser Arg Asn Gly Met Gln Pro Gln Ser Pro Thr Leu Ile Ala Leu Ala Ser Ser Lys Phe     124

      400         410         420         430         440         450         460         470
AAG ACT ATT AAT TTT AAT AAC AGT TCT CAA TCT ATT AAG AAT TGG AGT GCT CAG TCA AGG CGT GAG AAT CCA GTT TAT
Lys Thr Ile Asn Phe Asn Asn Ser Ser Gln Ser Ile Lys Asn Trp Ser Ala Gln Ser Arg Arg Glu Asn Pro Val Tyr     150

       480         490         500         510         520         530         540
GAA TAT AAA AAT CCT ATG GTC TTT GAA TAT AGA AAT TCA TAT ATT CTT CAA CGT GCT AAT CCA CAA TAT GGA AAC GTC
Glu Tyr Lys Asn Pro Met Val Phe Glu Tyr Arg Asn Ser Tyr Ile Leu Gln Arg Ala Asn Pro Gln Tyr Gly Asn Val     176

         560         570         580         590         600         610         620
ATG GGA TTG AGG TAT TAT ACA GCT AGT AAT ACA TGT CAA CTT GCA GCT TTT GAC TCA ACA CTC GCT GAA AAT GCA CCA
Met Gly Leu Arg Tyr Tyr Thr Ala Ser Asn Thr Cys Gln Leu Ala Ala Phe Asp Ser Thr Leu Ala Glu Asn Ala Pro     202

630         640         650         660         670         680         690         700
AAT AAT ACT CAA CGT TTT ATT TAC AAT GGA AGA CTT AAG AGA CCA ATT TCT AAT GTT TTA ATG AAA ATT GAA GCT GGT
Asn Asn Thr Gln Arg Phe Ile Tyr Asn Gly Arg Leu Lys Arg Pro Ile Ser Asn Val Leu Met Lys Ile Glu Ala Gly     228

   710         720         730         740         750         760         770         780
GCT CCA AAT ATT AAT AAT TTG ACA ATC TTA CCT GAC CCA ACA AAT CAG ACG ACA TGG TTA TAT AAC CCA GAT CAG CTG
Ala Pro Asn Ile Asn Asn Leu Thr Ile Leu Pro Asp Pro Thr Asn Gln Thr Thr Trp Leu Tyr Asn Pro Asp Gln Leu     254

      790         800         810         820         830         840         850         860
ATG AAT GGA ACA TTC ACC ATT GAA TTT TAT AAT AAC GGT CAG CTA GTT GAC ATG GTC AGG AAC ATG GGA GTG GTC ACC
Met Asn Gly Thr Phe Thr Ile Glu Phe Tyr Asn Asn Gly Gln Leu Val Asp Met Val Arg Asn Met Gly Val Val Thr     280

       870         880         890         900         910         920         930
GTT AGA ACA TTT GAT TCT TAC AGA ATT ACT ATT GAT ATG ATA AGA CCA GCG GCT ATG ACT CAA TAT GTC CAG AGA CTT
Val Arg Thr Phe Asp Ser Tyr Arg Ile Thr Ile Asp Met Ile Arg Pro Ala Ala Met Thr Gln Tyr Val Gln Arg Leu     306

         950         960         970         980         990        1000        1010
TTC CCA CAA GGC GGA CCA TAT CCC TAC CAA GCT GCA TAT ATG CTG ACT TTA AGT ATA CTC GAT GCT ACT ACA GAA TCA
Phe Pro Gln Gly Gly Pro Tyr Pro Tyr Gln Ala Ala Tyr Met Leu Thr Leu Ser Ile Leu Asp Ala Thr Thr Glu Ser     332

           1030        1040        1050        1060        1070        1080        1090
GTA CTA TGT GAT TCA CAT TCA GTT GAC TAC TCA ATT GTT GCT AAC GTT AGA AGA GAC TCA GCA ATG CCA GCT GGA ACA
Val Leu Cys Asp Ser His Ser Val Asp Tyr Ser Ile Val Ala Asn Val Arg Arg Asp Ser Ala Met Pro Ala Gly Thr     358

  1100        1110        1120        1130        1140        1150        1160        1170
GTA TTT CAG CCA GGT TTT CCA TGG GAA CAA ACT CTA TCC AAC TAC ACT GTT GCT CAA GAG GAT AAT CTA GAA AGA CTT
Val Phe Gln Pro Gly Phe Pro Trp Glu Gln Thr Leu Ser Asn Tyr Thr Val Ala Gln Glu Asp Asn Leu Glu Arg Leu     384

     1180        1190        1200        1210      1220      1230      1240      1250      1260
CTA CTT GTT GCA TCC GTG AAG AGA ATG GTG ATG TAG ATAAGCTAGAGATCTAACCAATCTCTATGTGGACTACTCACCATGTAGCATGATT
Leu Leu Val Ala Ser Val Lys Arg Met Val Met  *                                                          395

  1270      1280      1290      1300      1310      1320      1330      1340      1350
CACGAATGGGTTTAGTCCATGCTTGCGTAGGGGCAAATGCGCATGACGTGGATGATCCCCAGGAGGATGAAATGTGAACTATGTGGCT
```

FIG. 2. Corrected nucleotide and deduced amino acid sequences of the porcine group C/Cowden rotavirus gene encoding the major inner capsid protein VP6. The underlined regions at the 5′ and 3′ termini correspond to the cDNA and PCR amplification primer sequences. The underlined nucleotides (positions 451, 478–479, and 1063–1065, inclusive) represent the corrections to the published sequence. *, Termination codon. The GenBank nucleotide sequence database Accession Number is M94157.

TABLE 1

COMPARISON OF THE VP6 NUCLEOTIDE AND PROTEIN SEQUENCE HOMOLOGY OF THE GROUP C ROTAVIRUSES

Species of origin	Strain	Homology (%) cDNA	Homology (%) Protein	Reference
Porcine	Cowden	100	100	
Human	Belém	83.4	91.6	
Human	Preston	84.0	91.6	
Human	Bristol	84.0	91.6	*15*

turers (Dynal A.S.), except 300 m*M* NaOH was used for the denaturation of the DNA. The adsorbed DNA was sequenced using a T7 polymerase (Sequenase version 2.0) dideoxynucleotide DNA sequencing system (Cambridge BioScience) with deoxyadenosine 5′-[α^{35}S]triphosphate (Amersham).

The porcine group C/Cowden VP6 cDNA was reported to be 1349 nucleotides containing a coding region of 1182 nucleotides (394 amino acid; deduced M_r 44,479 Da) and was assigned to segment 6 of the genome (*14*), which compared favorably with the group A rotavirus VP6 coding assignment. However, it would appear that segment 5 encodes the VP6 protein (*15*) and that segment 6 encodes the NS34 equivalent of the group A rotaviruses (*20*).

Previous sequence alignments between the Bristol and Cowden VP6 proteins revealed a region of 10 amino acids that was significantly different (*15*). Direct sequencing of PCR-amplified cDNA of the Cowden VP6 gene demonstrated an extra three bases within this region of divergence (Fig. 1). This dramatically altered the corresponding protein sequence and effectively eliminated the sequence differences. Sequencing of the complete Cowden VP6 gene revealed three further changes (nucleotide positions 1063–1065 inclusive). These three changes corresponded to one amino acid change from a tryptophan residue in the published sequence to a valine residue (Fig. 4, amino acid position 348), thereby eliminating another difference between the porcine and human proteins. The corrected Cowden VP6 gene (Fig. 2) comprised 1352 nucleotides with an open reading frame of 1185 nucleotides (395 amino acids; deduced M_r 44,614).

The nucleotide differences between the two Cowden VP6 genes suggested that the sequence reported here is the correct sequence for the gene. However it is not known whether the Cowden isolate used is the same as that used previously (*14*), and the possibility that the two Cowden strains had diverged in some way cannot be eliminated, but is extremely unlikely. The original Cowden VP6 nucleotide sequence (*14*) was obtained from two cDNA clones and the sequence reported here was determined from a PCR-amplified cDNA pool. It is possible that the errors within the original sequence arose during the cDNA synthesis, whereas the corrected sequence reported here directly corresponds to the true genomic sequence.

The corrected nucleotide and deduced amino acid sequences of the porcine group C/Cowden rotavirus VP6 gene demonstrated a high degree of nucleotide and protein sequence homology (Table 1) with the human group C/Bristol rotavirus VP6 gene (84.0 and 91.6%, respectively).

The nucleotide sequence of the human group C/Bristol VP6 gene was originally determined from two M13 bacteriophage clones of opposite polarity containing PCR-amplified cDNA (*15*). Direct sequencing of PCR-amplified VP6 cDNA from the Bristol strain (data not shown) confirmed that there were no changes within the reported nucleotide sequence of the cloned VP6 gene. However, a recent cloning technique (*19*) has allowed the terminal nucleotide sequences of the Bristol VP6 gene to be determined. The previously reported (*15*) 5′ terminal sequence (5′-GCATTTAAAATCT-3′) corresponded to the 5′ terminal sequence of the porcine group C/Cowden VP6 gene and was incorporated into a primer used for the reported cDNA synthesis of the human VP6 gene (*15*). The correct 5′ terminal sequence of the human group C/Bristol VP6 gene (*19*) comprised 5′-GGCTTTAAAAATCT-3′ and revealed three nucleotide differences in the first 15 bases, including an extra nucleotide (A) at position 11. The 5′ terminal sequence GGC has been found in all group A genome segments (*4*) and the group C segments we have sequenced to date (*19*). The 5′ noncoding sequence of the human group C/Bristol VP6 gene was thereby increased to 22 nucleotides.

The complete human group C/Belém and C/Preston VP6 nucleotide sequences of 1353 bases each contained an open reading frame of 1185 bases (395 amino acids; deduced M_r 44,669). Analysis of the sequences revealed a 5′ noncoding region of 22 nucleotides and a 3′ noncoding region of 146 nucleotides (Fig. 3) within each gene. By comparison the Belém and Preston VP6 genes are of an equivalent size to the human group C/Bristol VP6 gene (*15*).

The nucleotide and deduced amino acid sequences of the novel human group C rotavirus VP6 genes demonstrated a high degree of nucleotide and protein sequence homology (Table 1) with the corrected porcine group C/Cowden rotavirus VP6 gene (83.4 and 91.6%, respectively, for the group C/Belém rotavirus; 84.0 and 91.6%, respectively, for the group C/Preston rotavirus). In comparison the VP6 genes of both strains demonstrated a high degree of nucleotide and protein

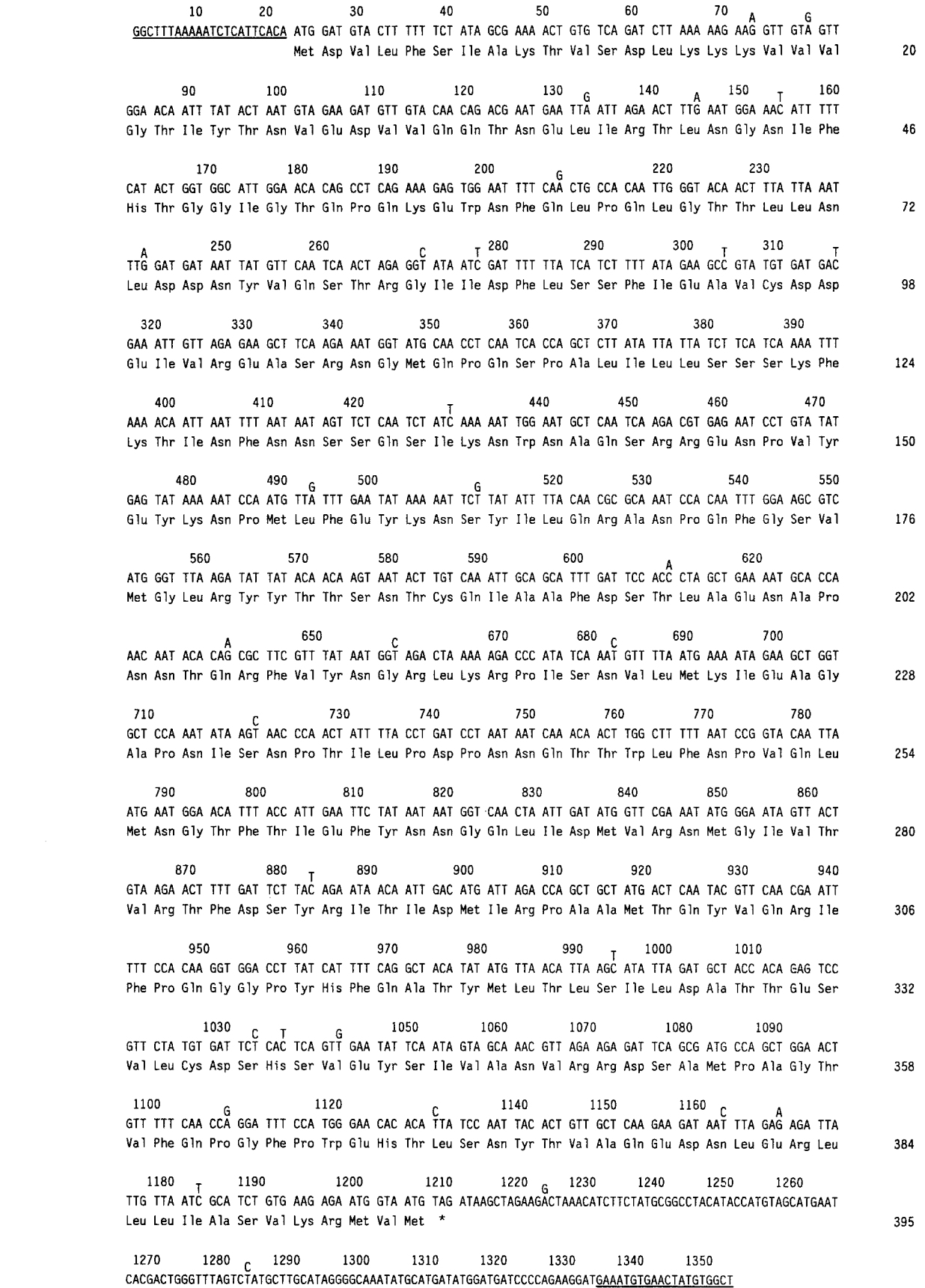

FIG. 3. Nucleotide and deduced amino acid sequence of the human group C/Belém and C/Preston rotavirus genes encoding the major inner capsid protein VP6. The underlined regions correspond to the cDNA and PCR amplification primer sequences. The overlying nucleotide changes indicate the differences within the Preston VP6 gene. *, Termination codon. The GenBank database Accession Numbers for the human group C/Belém and the human group C/Preston VP6 nucleotide sequences are M94155 and M94156, respectively.

```
      10        20        30        40        50        60        70        80        90
MDVLFSIAKTVSDLKKKVVVGTIYTNVEDIIQQTNELIRTLNGNTFHTGGIGTQPQKEWNFQLPQLGTTLLNLDDNYVQATRSVIDYLASFIEAVC
.............................VV.............I................................S..GI..F.S.......
.............................VV.............I................................S..GI..F.S.......
.............................VV.............I................................S..GI..F.S.......

 100       110       120       130       140       150       160       170       180       190
DDEIVREASRNGMQPQSPTLIALASSKFKTINFNNSSQSIKNWSAQSRRENPVYEYKNPMVFEYRNSYILQRANPQYGNVMGLRYYTASNTCQLAA
..................A..L.S....................N................L...K...........F.S........T.....I..
..................A..L.S....................N................L...K...........F.S........T.....I..
..................A..L.S....................N................L...K...........F.S........T.....I..

     200       210       220       230       240       250       260       270       280
FDSTLAENAPNNTQRFIYNGRLKRPISNVLMKIEAGAPNINNLTILPDPTNQTTWLYNPDQLMNGTFTIEFYNNGQLVDMVRNMGVVTVRTFDSYR
................V.......................S.P......N......F..V.................I.......I..........
................V.......................S.P......N......F..V.................I.......I..........
................V.......................S.P......N......F..V.................I.......I..........

         300       310       320       330       340       350       360       370       380
ITIDMIRPAAMTQYVQRLFPQGGPYPYQAAYMLTLSILDATTESVLCDSHSVDYSIVANVRRDSAMPAGTVFQPGFPWEQTLSNYTVAQEDNLERL
................I.......HF..T.....................E.........................H..................
................I.......HF..T.....................E.........................H..................
................I.......HF..T.....................E.........................H..................

   390
LLVASVKRMVM               group C/Cowden
..I........               group C/Belém, Brazil
..I........               group C/Preston, U.K.
..I........               group C/Bristol, U.K.
```

FIG. 4. Comparison of the VP6 protein sequences of the group C/Belém, C/Preston, C/Bristol, and C/Cowden rotaviruses. Sequence homologies are indicated by dots. The corrected group C/Cowden sequence is shown.

sequence homology with the human group C/Bristol rotavirus VP6 gene (97.9 and 100%, respectively, for the group C/Belém rotavirus; 99.6 and 100%, respectively, for the group C/Preston rotavirus).

Comparison of the human group C/Belém and C/Bristol VP6 nucleotide sequences revealed 28 base changes of which 25 occur within the coding sequence. By contrast, comparison of the Belém VP6 nucleotide sequence with the Cowden sequence revealed 226 base changes in total. Remarkably each of the 25 changes between the two human VP6 coding sequences were silent and did not alter the predicted VP6 protein sequence (Fig. 3). This is even more surprising considering the geographical separation of the two isolates.

Comparison of the human group C/Preston and C/Bristol VP6 nucleotide sequences revealed five base changes, of which four occur within the coding sequence. Again, each of the four changes between the two human VP6 coding sequences were silent and did not alter the predicted VP6 protein sequence (Fig. 3).

As the results were obtained from the direct sequencing of PCR-amplified cDNA of the VP6 gene from four rotavirus isolates, the possibility existed that the high conservation of the VP6 proteins observed was due to the amplification and sequencing of a common contaminant. This possibility has been eliminated because the nucleotide sequences of the four VP6 genes were different. If a common contaminant had been sequenced then the nucleotide sequences obtained would have been identical.

The results show that human isolates from different continents of the world share identical VP6 proteins. The extreme conservation among the VP6 proteins of the human isolates provides invaluable information to aid the development of a diagnostic test for the detection of group C rotaviruses. Further sequence studies of the major inner capsid protein will reveal whether subgroups exist within the group C rotaviruses as within the group A rotaviruses.

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