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Molecular characterization of the genotype G9 human rotavirus strains recovered in Palermo, Italy, during the winter of 1999–2000

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SUMMARY

Among the known human rotavirus serotypes, types G1–G4 are ubiquitous and account for >80% of global human rotavirus strains. Since 1994 an increase in reports of G9 serotype isolates has been observed in both developed and developing countries. In the winter season of 1999–2000 we detected the appearance of G9 rotavirus in Palermo, Italy, accounting for 19% of all rotaviruses circulating in our geographical area. Some of these Italian G9 isolates have been submitted to partial sequencing of their VP7 gene. All of them showed complete nucleotide identity suggesting a clonal origin. The Italian VP7 sequences were found to be very closely related to that of other G9 strains recently isolated in Europe, America, Africa and Asia. Our results confirm that G9 strains circulating worldwide since 1994 are closely related genetically in their VP7 genes.

INTRODUCTION

Human group A rotaviruses (HRV) are the leading aetiological agent of severe acute diarrhoea in infants and young children worldwide and efforts are currently under way to develop a vaccine [1]. HRV strains exhibit wide genetic and antigenic heterogeneities and, on the basis of the two outer capsid proteins, VP7 and VP4, both involved in neutralizing activities, they have been classified into 10 G types and 11 P types respectively [2]. Sequence analysis has also revealed the existence of genetic lineages within the G and P types [3, 4]. As with influenza viruses, reassortment of co-circulating viruses and sequential point mutations seem to be the most important causes for genetic variation [5, 6].

Previous epidemiological studies have shown that G1, G2, G3 and G4 serotypes are ubiquitous and that together they account for >80% of globally isolated human rotavirus strains. Therefore, protection against them is seen as a primary target for vaccine development. However, the increase in reports of other rotavirus serotypes as a cause of severe diarrhoea suggests that any future vaccine may need to incorporate additional specificities [7]. Since 1994 serotype G9 rotavirus has been increasingly reported in both developed and developing countries, raising the concern that this G type is of emerging importance as a human pathogen [8, 9]. Genetic and antigenic characterization and the phylogenetic analysis of the VP7 of rotavirus strains with G9 specificity have provided information about the origin of such strains and their evolution [10]. Within Europe, the G9 serotype has been documented in England since 1995, in France and Ireland from 1997 to 1998, and in The Netherlands from 1999 [11–15]. The first report of a high incidence of G9-type gastroenteritis occurred in Italy during the winter of 1999–2000 [16].

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Table. *Sequence comparison between Italian G9 rotavirus isolates (nucleotides 82–983, amino acids 12–312) and the VP7 gene of other representative human G9 rotaviruses*

Continent/country (city)	Strain/accession no.	Time period	Homology (%) nt	aa
America				
USA (Philadelphia)	WI61/NR*	1983	89·7	96
USA (Philadelphia)	US1145/AJ250270	1995	99·1	99·3
USA (Indianapolis)	US1212/AJ250272	1996–1997	98·7	98·3
USA (Indianapolis)	US1205/AF060487	1996–1997	99·1	99·6
USA (Kansas City)	US1071/AJ250268	1996–1997	99·1	99·3
USA (Omaha)	US1343/AJ250273	1996–1997	99	99·6
USA (Cincinnati)	US244/AJ250274	1997–1998	99·1	99·3
Brazil (Rio de Janeiro)	R1527/AJ279082	1997–1998	99·6	99·6
Brazil (Rio de Janeiro)	R136/AF438228	1998	99·7	99·6
Brazil (Rio de Janeiro)	R146/AF274970	1999	99·3	99·6
Brazil (Rio de Janeiro)	R160/AF274971	1999	99·5	99·3
Asia				
India	116E/L14072	1985	83	86·3
India	INL1/AJ250277	1994	97·5	96·3
India	ING16/AJ250276	1994	97·5	96·3
Bangladesh	BD524/AJ250543	1995–1996	97·6	96·3
Bangladesh	BD426/AJ250541	1995–1996	97·8	97
Bangladesh	BD431/AJ250542	1995–1996	98	97
Japan	F45/NR*	1987	89·2	95·6
Japan	95H115/AB045373	1994–1995	99·4	100
Japan	TK2082/AB091755	1999	94·4	97·3
Thailand (Chiang Mai)	Mc323/D38053	1989	97	98
China	T203/AY003871	1997	94·8	97·3
China	97′SZ37/AF260959	1997	89·6	96·6
Africa				
Nigeria	Bulumkutu/AF359358	1999–2000	99	99·3
Malawi (Blantyre)	MW47/AJ250544	1997	99·2	99·6
South Africa	4330LC/AF529865	1998	98·3	98·6
South Africa	6342LP/AF529872	1999	98·7	98·3
Europe				
UK (Birmingham)	480/AJ401261	1996–1997	98·5	98
UK (London)	0.7/AJ401239	1997–1998	99·1	99

* NR, Not recorded in on-line databases. Sequences obtained from Green et al. [20].

During this season, G9 strains constituted 19% of all rotaviruses identified and they represented the third most common type circulating in the paediatric population of Palermo, Italy.

In this paper we report the results of the nucleotide and deduced amino-acid sequence analyses of VP7 gene products of six Italian G9 isolates.

METHODS

VP7 gene products of six Italian G9 isolates, representative of the sampling period, were randomly selected from rotavirus strains collected in the Palermo study (August 1999–July 2000). All the isolates had previously been classified as G9 by reverse transcription (RT)–PCR, using primers specific for genotypes G1–G4 and G9 described by Gouvea et al. [17]; these isolates shared the same genotypic properties: P[8] genotype, subgroup II, and long electropherotype [16].

Amplicons generated in the first round of the G-typing RT–PCR [17] were used to obtain partial gene 9 (encoding VP7) DNA sequences (MWG Biotech, Ebersberg, Germany). Alignments were performed

using Clustal W software, and the phylogenetic analysis was carried out with the VP7 sequences (nucleotides 82–983) and deduced amino-acid sequences (amino acids 12–312) using the MEGA software version 2.1 [18, 19]. The statistical significance of the phylogenies constructed was estimated by means of bootstrap analysis with 100 pseudoreplicate data sets.

The partial VP7 sequences of the Italian G9 isolates (IT123, IT379, IT397, IT400, IT413, IT624) were analysed and compared with each other and with other similar G9 rotavirus VP7 published sequences.

VP4-specific amplicons of 876 bp (spanning nucleotides 11–887) from the six Italian isolates were submitted to partial sequencing of the VP4 gene [16].

RESULTS

The sequences of the VP7 encoding genes of our isolates were 100% identical and confirmed their attribution to the G9 genotype as they had >89% nucleotide and >96% deduced amino-acid sequence identity with the VP7 genes of strains WI61 and F45 (see Table), which are the reference strains for the G9 genotype [20]. The phylogenetic analysis of the nucleotide sequences revealed that the Italian isolates clustered with the main G9 lineage, including recently recovered European, American, African and Asiatic G9 strains (Fig. 1). WI61 and F45 strains clustered together while separate lineages were formed by the Indian strain 116E, Chinese strains 97′SZ37 and T203, Japanese strain TK2082 and strain Mc323 from Thailand.

Both the nucleotide and deduced amino-acid sequence of the Italian isolates were more closely related to G9 strains recently recovered in the United Kingdom, America and Africa. The percentage identity of Asian strains was generally lower, with the exception of strain 95H115 isolated in Japan in 1994–1995, which showed 99·4% nucleotide and 100% amino-acid identity. Analysis of the deduced amino-acid sequences in the antigenic regions A, B, C, D and F of the VP7 gene compared to the reference strains WI61 and F45 revealed 6 and 5 substitutions respectively. In both cases, a single amino-acid substitution occurred in regions A and F at positions 87 and 242 and a double substitution in region C at positions 208 and 220. Two substitutions were present in region D at positions 73 and 74, with respect to WI61, and a single substitution at position 70 with respect to F45. Italian G9 isolates were identical to strains MW47 (Malawi), R146 (Brazil), US1205, US1212, US1145, US1343 and US244 (United States), 95H115 (Japan), and 0.7 (United Kingdom) in all antigenic regions (A–F) of the VP7 gene (Fig. 2).

Analysis of partial VP4 gene sequences of the G9 Italian isolates confirmed their perfect identity and revealed a high degree of nucleotide and amino-acid sequence similarity (>99%) to the VP4 of the G1 Malawian strain OP351 (data not shown).

DISCUSSION

G9 strains were first identified in the human population in the United States in 1983 [21], but they were not reported again until more than a decade later [8]. Genetic analysis has shown that the more recently recovered G9 strains (in the 1990s) did not directly evolve from those found in the previous decade, but that they represented an independent introduction of novel G9 strains [10, 13].

G9 strains in Italy were first reported in 1997 [22], but a high incidence of G9 rotavirus gastroenteritis occurred only in the winter of 1999–2000 [16]. A retrospective study carried out by RT–PCR on untypable rotavirus isolates collected in Palermo from 1995 to 1999 failed to identify any other G9 strains.

Partial sequencing of the VP7 gene revealed the complete identity of the Italian G9 isolates collected during the winter of 1999–2000 and their close relationship with the rotaviruses which have been circulating worldwide since the mid-1990s. Remarkably, the VP7 amino-acid sequences of our isolates were indistinguishable from that of the Japanese 95H115 strain isolated in 1994–1995 [23]. The latter strain is the earliest of the globally re-emerging G9 human rotaviruses and it is considered as a candidate donor strain of the G9 VP7 gene to United States G9 rotavirus strains [10]. Unfortunately, the G9 rotaviruses recovered in Italy in 1994 were no longer available and could not be examined by genetic analysis.

The rotavirus G9 type has been found to be associated with the VP4 genotypes P[6], P[8], P[11], or P[19] [6, 9, 11, 24]. In Europe G9 strains with P[6] specificity were first reported in 1995–1996 in the United Kingdom, where subsequently they were displaced by G9P[8] strains [13]. The latter, which probably originated by reassortment with co-circulating rotaviruses, were also detected in France in 1997–1998 and in Ireland in 1998 [11, 14]. Genetic analysis of the VP7 gene of Italian G9 isolates showed 99% amino-acid identity with G9P[8] UK strains. Other genotypic properties, such as subgroup II and long

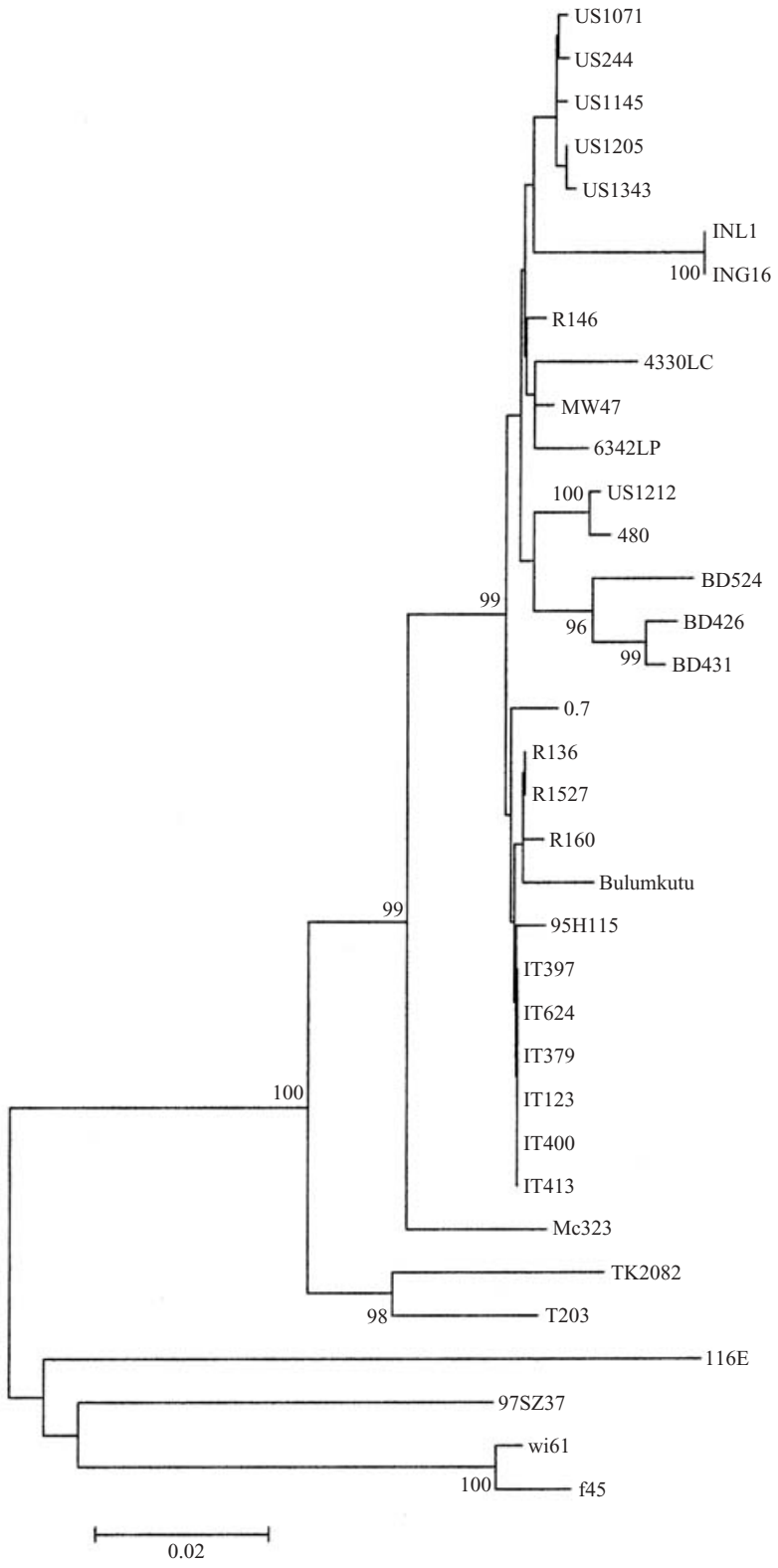

Fig. 1. Phylogenetic tree constructed from 902-bp cDNA fragments of the VP7 genes of 6 Italian and 29 other human rotavirus strains by using the Clustal W and MEGA software and the neighbour-joining method. Significant bootstrap values (>90%) are shown at the branching point of each cluster. The scale bar is proportional to genetic distance. Sources of sequences are as indicated in the Table.

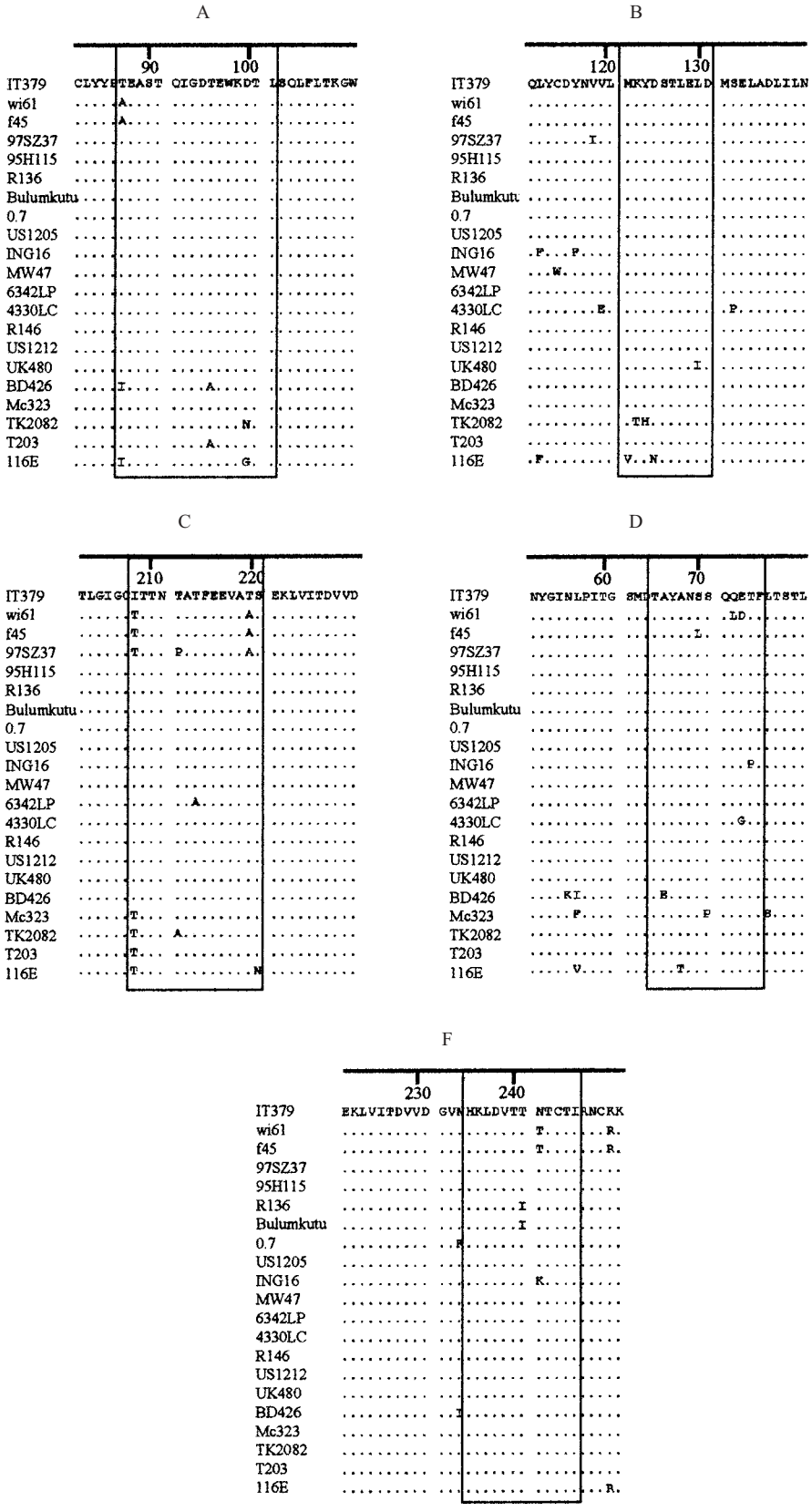

Fig. 2. Deduced amino-acid sequences of the antigenic regions A–D and F of IT379 Italian isolate compared to sequences of representative G9 human rotavirus strains.

electropherotype, were present in both groups of strains, further supporting a close genetic relationship. A clonal origin of the G9 Italian isolates can be supported by their VP4 gene complete identity. The high degree of homology between the VP4 nucleotide sequences of our G9 isolates and the G1 Malawian strain OP351 further supports the hypothesis that the G9P[8] strains have emerged through reassortment in humans between G9 strains introduced recently and the prevalent co-circulating G1, G3 and G4 commonly carrying VP4 genes of P[8] type. It has been suggested that strong interaction between G9 VP7 and other structural rotavirus proteins is important for allowing stable G9 strains to thrive in the community and spread extensively across various continents [9].

In conclusion, our study contributes to reinforcing the need to implement a continuous surveillance system for rotavirus infections at several locations in order to monitor the spread of emerging strains properly. Vaccine strategies will have to take into account the evolution of strains.

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