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Clustering of mumps virus isolates by SH gene sequence only partially reflects geographical origin

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Summary. The nucleotide sequences of the SH gene of 45 new mumps virus isolates derived from different parts of Europe, Canada and USA were determined. A phylogenetic tree was constructed which confirmed the existence of three major clusters. While clustering according to geographical origin was clear in some cases each of the major clusters included isolates which were widely separated in origin. The degree of variability between the sequences of SH genes of different strains and the ratio of coding to non coding differences were both very high compared to those observed in other genes such as the M and HN. A standardized system of nomenclature of mumps strains was established.

Introduction

Mumps virus is a negative sense, non-segmented RNA virus which belongs to the *Rubulavirus* genus of the subfamily *Paramyxovirinae* [16]. The order of the genes in the genome is: 3′-leader-N-V/NS2/P-M-F-SH-HN-L-trailer-5′ [6]. Of these the nucleocapsid (N), phospho (P), matrix (M) and the large (L) proteins are classified as the core proteins while the fusion (F) and the haemagglutinin-neuraminidase (HN) are envelope coat proteins of the virion. The mRNAs of NS2 and P genes are generated from the V/NS1 non-structural protein gene by a nucleotide editing mechanism [9].

The SH gene of mumps virus is 316 nucleotides in length. The nucleotide sequence of this region is highly variable between strains and has been used to distinguish vaccine and wild type viruses and to establish genetic lineages between mumps isolates [2, 14, 15, 18, 25]. However the gene product of the SH gene has never been identified and it may not be translated.

In this study, we report the nucleotide sequences of 45 new isolates from different parts of Europe, Canada and USA. By using sequence data generated during this study and elsewhere a new phylogenetic tree has been constructed and analysed. The tree is very similar to those based on sequences of other genes

and reveals a number of lineages, at least some of which are composed of geographically clustered isolates.

Materials and methods

Viruses and nomenclature

All mumps virus isolates for which the SH gene sequence is available now are reported in Table 1. After consultation with groups currently working on mumps virus a new nomenclature is proposed in which the name given to an isolate is now followed by the country and year of original isolation. The isolates designated as Ca6.2/Canada/88 and Gel/Germany/90 were derived respectively from a monkey and a child following immunisation with mumps vaccine of the Jeryl Lynn strain. Strain Ge2/Germany/87 was isolated from a human subject after immunisation with the Russian L-3 vaccine strain. The Nt5/UK/90 and Ed7/UK/89 were isolated following vaccination with the Urabe strain of mumps vaccine. The isolate described as RG [4] was originally isolated in London in 1991 and therefore labelled as Lo18/UK/91 during this study. All German isolates studied were received from Prof. S. Dittmann, Institute for Virology and Epidemiology, Berlin. The French isolates were received from Prof. M. Aymard, Laboratorie de Virologie Centre Hospitalo, Universite Claude Bernard, Lyon. The Enders strain sequenced at NIBSC, designated as End2, was obtained from Prof. B. K. Rima (Belfast, UK). The Euro 1 isolate grew out as a contaminant of another preparation of Enders. Its origin is unknown. Except for post vaccination isolates, details of the epidemiological relationships between the strains and their isolation and passage are mostly incomplete. All isolates other than commercial vaccines had been passaged at least two to three times in Vero cells before being used for sequencing at NIBSC.

RNA extraction

The RNA was extracted from virus infected tissue culture fluid and/or vaccine material either by the method of proteinase-K digestion followed by phenol-chloroform extraction or by using our recently established protocol of Instant RNA extraction [1].

RT-PCR

The RNA was reverse transcribed into cDNA with AMV reverse transcriptase at 42 °C in the presence of random primers (dN6) and other essential reagents. The cDNA was subsequently amplified by Taq polymerase in the presence of a set of primers (P1/P2) as described earlier [2]. The amplified PCR products were purified by a prep-a-gene (Bio-Rad) in 15 μl H_2O, of which 4 μl was used for direct PCR sequencing [3]. Primer 4 889 (5′-AGTTTCGAG-GGCTCCAT-3′ positions 397–413) [2] was used to determine the genome sense sequence while the second primer 4 890 (GTCGATGATCTCATCAGGTAC-3′ positions 2 984–3 004 [8]) was used to generate the message sense sequence. Sequence data were analysed by the GCG Version 8.1 computer software.

Results

Of the 318 nucleotides sequenced, 1–316 belonged to the SH gene and 317–318 formed the SH-HN intergenic region. Forty-five isolates were examined from which thirty one unique SH gene sequences were generated. The German isolates

Table 1. The description of mumps virus isolates

Virus isolate	Description	Original isolation	Proposed nomenclature	SH gene sequence	Virus source to NIBSC
Canada					
Ca1.3	Canada 1.3	1987	Ca1.3/Canada/87	This study	Vac. manu. 1989
Ca1.7	Canada 1.7	1985	Ca1.7/Canada/85	〃 〃	〃 〃 〃
Ca7	Canada 7	1988	Ca7/Canada/88	〃 〃	〃 〃 〃
Ca7.1	Canada 7.1	1987	Ca7.1/Canada/88	〃 〃	〃 〃 〃
Ca8	Canada 8	1988	Ca8/Canada/88	〃 〃	〃 〃 〃
Denmark					
De1	Denmark 1	1983	De1/Denmark/83	This study	Vac. manu. 1989
De2	Denmark 2	1984	De2/Denmark/84	〃 〃	〃 〃 〃
France					
F2	France 2	1990	F2/France/90	This study	Prof. M. Aymard 1990
F3	France 3	1989	F3/France/89	〃 〃	〃 〃 〃
F5	France 5	1987	F5/France/87	〃 〃	〃 〃 〃
F6	France 6	1989	F6/France/89	〃 〃	〃 〃 〃
F15	France 15	1989	F15/France/89	〃 〃	〃 〃 1992
Germany					
Bln 77	Berlin 77	1977	Bln 77/Germany/77	[15]	
Bln 92.1	Berlin 92.1	1992	Bln 92.1/Germany/92	[14]	
Bln 92.2	Berlin 92.2	1992	Bln 92.2/Germany/92	[14]	
Ch 5/87	Chemnitz 5	1987	Ch 5/Germany/87	[15]	
Ch 7/87	Chemnitz 7	1987	Ch 7/Germany/87	[15]	
Ge 9	Germany 9	1977	Ge 9/Germany/77	This study	Prof. S. Dittmann 1990
Ge 10	Germany 10	1977	Ge 10/Germany/77	〃 〃	〃 〃 〃
Ge 11	Germany 11	1977	Ge 11/Germany/77	〃 〃	〃 〃 〃
Ge 12	Germany 12	1977	Ge 12/Germany/77	〃 〃	〃 〃 〃
Ge 13	Germany 13	1977	Ge 13/Germany/77	〃 〃	〃 〃 〃
Ha 92	Halle 92	1992	Ha 92/Germany/92	[15]	
Hi 93	Hildesheim 93	1993	Hi 93/Germany/93	[14]	
Hv 90	Hannover 90	1990	Hv 90/Germany/90	[14]	
Hv 87	Hannover 87	1987	Hv 87/Germany/87	[14]	

(*Continued*)

Table 1 (*continued*)

Virus isolate	Description	Original isolation	Proposed nomenclature	SH gene sequence	Virus source to NIBSC
Japan					
Ja 6	Japan 6	1989	Ja 6/Japan/89	This study	Vac. manu. 1991
Ja 7	Japan 7	1989	Ja 7/Japan/89	″ ″	″ ″ ″
Miy	Miyahara	1970 [20]	MIY/Japan/70	[19] D90234	
Tak	Takahashi	1977 [20]	Tak/Japan/77	[19] D90235	
Ur	Urabe (wt)	1967 [22]	Ur/Japan/67	[19] D90236	
Ur	Urabe (vac)	1973 [23]		[2]	Vac. manu. 1990
Russia					
L3	Leningrad 3	1953 [17]	L3/Russia/53	[15]	
Sweden					
SBL1	SBL1	1969 (Örvell)[a]			
SBL1/e	SBL1/egg		Sb11/Sweden/69	[8]	
SBL1/v	SBL1/Vero			[7]	
Switzerland					
Mo 92	Moutier 92	1992	Mo 92/Switzerland/92	[18] U35848	
Sw 93.1	Switzerland 93.1	1993	Sw 93.1/Switzerland/93	[14]	
Sw 93.2	Switzerland 93.2	1993	Sw 93.2/Switzerland/93	[14]	
Rub	Rubini	1974 (Glück)[a]	Rub/Switzerland/74	[14] X72944, This study	Vac. manu. 1993
YLB92	Yuerdon L. Bains 92	1992	YLB92/Switzerland/92	[18] U35847	
YLB 95	Yuerdon L. Bains 95	1995	YLB 95/Switzerland/95	[18] U35849	
UK					
Be 1	Bedford 1	1989	Be 1/UK/89	This study	P.H.L. UK. 1989
Bf	Belfast	1975	Bf/UK/75	[25]	
Bm 1	Birmingham 1	1990	Bm 1/UK/90	This study	P.H.L. UK. 1990
Bm 2	Birmingham 2	1990	Bm 2/UK/90	″ ″	″ ″ ″
Bs 1	Bristle 1	1989	Bs 1/UK/89	[25]	″ ″ 1989
Do 1	Dorset 1	1988	Do 1/UK/88	This study	″ ″ ″
Do 2	Dorset 2	1988	Do 2/UK/88	″ ″	″ ″ ″
Do 4	Dorset 4	1988	Do 4/UK/88	″ ″	″ ″ ″

(*Continued*)

Table 1 (*continued*)

Do 6	Dorset 6	1987	Do 6/UK/87	″ ″	″ ″ ″
Do 9	Dorset 9	1987	Do 9/UK/87	″ ″	″ ″ ″
Ed 2	Edinburgh 2	1988?	Ed 2/UK/88	[25]	
Ed 4	Edinburgh 4	1988?	Ed 4/UK/88	[25]	
Ed 6	Edinburgh 6	1988?	Ed6/UK/88	[25]	
Ed 2.2	Edinburgh 2.2	1988	Ed 2.2/UK/88	This study	R.V.L. Edinburgh 1988
Ed 4.3	Edinburgh 4.3	1988	Ed 4.3/UK/88	″ ″	″ ″ ″
Ed 6.3	Edinburgh 6.3	1988	Ed 6.3/UK/88	″ ″	″ ″ ″
Euro 1	Europe 1	?		″ ″	
Lo 1	London 1	1988	Lo 1/UK/88	″ ″	Dr. P.D. Minor 1988
Lo 2	London 2	1987	Lo 2/UK/87	″ ″	R.P.M.S. London 1989
Lo 18 (RG)	London 18	1991 [4]	Lo 18/UK/91	″ ″	St. G.H. London 1992
Nt 2	Nottingham 2	1989	Nt2/UK/89	″ ″	P.H.L. UK. 1989
USA					
Cal 1	California 1	1968	Cal 1/USA/68	This study	Vac. manu. 1989
Cal 2	California 2	1971	Cal 2/USA/71	″ ″	Vac. manu. 1989
End	Enders	1945 [10]	End/USA/45	[19, 25] D90231	
End 2	Enders 2			This study	Prof. B.K. Rima 1994
Jl	Jeryl Lynn	1963 [5]		[19] D90232	
Jl 2	Jeryl Lynn 2			[2]	Vac. manu. 1990
Jl 5	Jeryl Lynn 5			[2]	″ ″ ″
Kil	Kilham	1950 [13]	Kil/USA/50	[25]	
Rw	RW		Rw/USA/	[25]	
Post-vaccine isolates					
Ca 6.2	Canada 6.2	1988	Ca 6.2/Canada/88	This study	Vac. manu. 1989
Ed 7	Edinburgh 7	1989	Ed 7/UK/89	″ ″	R.V.L. Edinburgh 1989
Ge 1	Germany 1	1990	Ge 1/Germany/90	″ ″	Prof. S. Dittmann 1990
Ge 2	Germany 2	1987	Ge 2/Germany/87	″ ″	″ ″ ″
Mat	Matsuyama	1984 [21]	Mat/Japan/84	[19] D90233	
Nt 5	Nottingham 5	1990	Nt 5/UK/90	This study	P.H.L. UK. 1990

P.H.L. Public Health Laboratory. *R.V.L.* Regional Virus Laboratory. *R.P.M.S.* Royal Postgraduate Medical School. *St. G.H.* St. George Hospital. *Vac. manu.* Vaccine manufacturer. This study, see accession no. DS 26771

[a] Personal communication

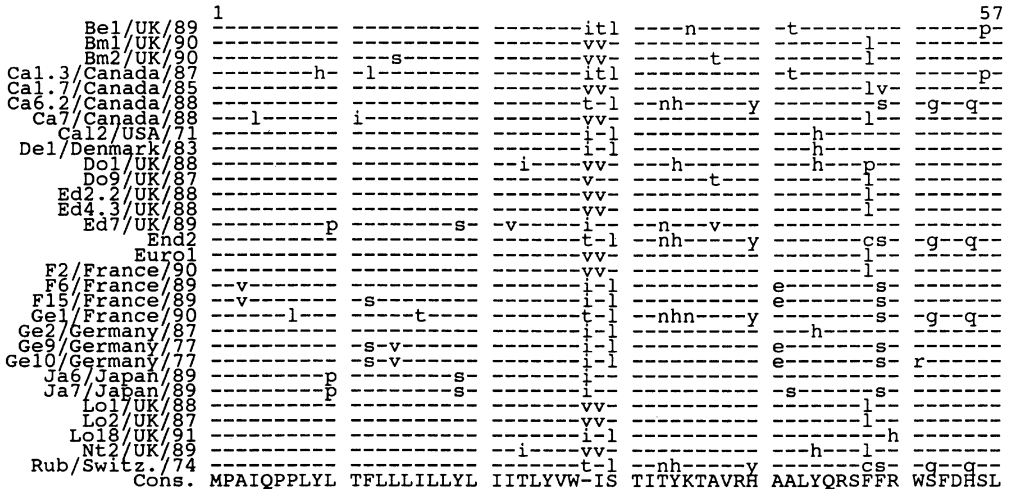

Fig. 1. Alignment of deduced amino acid sequences of the SH genes

Ge11/Germany/77, Ge12/Germany/77, Ge13/Germany/77 and Ge19/Germany/77 were identical in sequence to Ge9/Germany/77. The French isolates F3/France/89 and F5/France/87 were identical to F2/France/90, and the Danish isolates Del/Denmark/83 and De2/Denmark/84 were identical to the American isolate Cal1/USA/68. The English isolates Do1/UK/88 and Do2/UK/88 were identical, and the English isolates Do4/UK/88 and Bm1/UK/90 were identical to the Canadian isolate Ca8/Canada/88. Finally the Canadian isolates Ca1.3/Canada/87 and Ca7.1/Canada/87 were identical, the Scottish strains Ed2.2/UK/88 and Ed6.3/UK/88 were identical, and the English strain Do6/UK/87 was identical to the Scottish strain Ed4.3/UK/88.

The alignment of 31 unique nucleotide sequences generated along with our three published sequences [2] has been lodged with the EMBL nucleotide sequence database (accession number DS 26771). The hypothetical amino acid sequences translated from the new sequences, except Nt5/UK/90, are shown in Fig. 1. A dendrogram of the new and published nucleotide sequences was constructed by the growtree programme of GCG software with UPGMA (unweighted pair group method using arithmetic averages) and is shown in Fig. 2. The isolates formed three clusters A, B and C, consistent with previous reports based on sequences of the M, F, SH and HN genes [3, 11, 14, 15, 24, 25].

Group C consisted of 34 viruses of which 31 were of European origin and three Canadian. The European isolates were from the United Kingdom, Germany, Switzerland and France. The sequences published for the Scottish isolates Ed2, Ed4 and Ed6 [25] form a sublineage designated C1 in Fig. 2, distinct from the sublineage C7 which includes viruses Ed6.3/UK/88, Ed4.3/UK/88 and Ed2.2/UK/88. According to their passage history, Ed2.2/UK/88 was only one passage from Ed2, while Ed4.3/UK/88 and Ed6.3/UK/88 were only two passages from Ed4 and Ed6 respectively and they would therefore be expected to be closely related. Moreover when inocula of Ed2

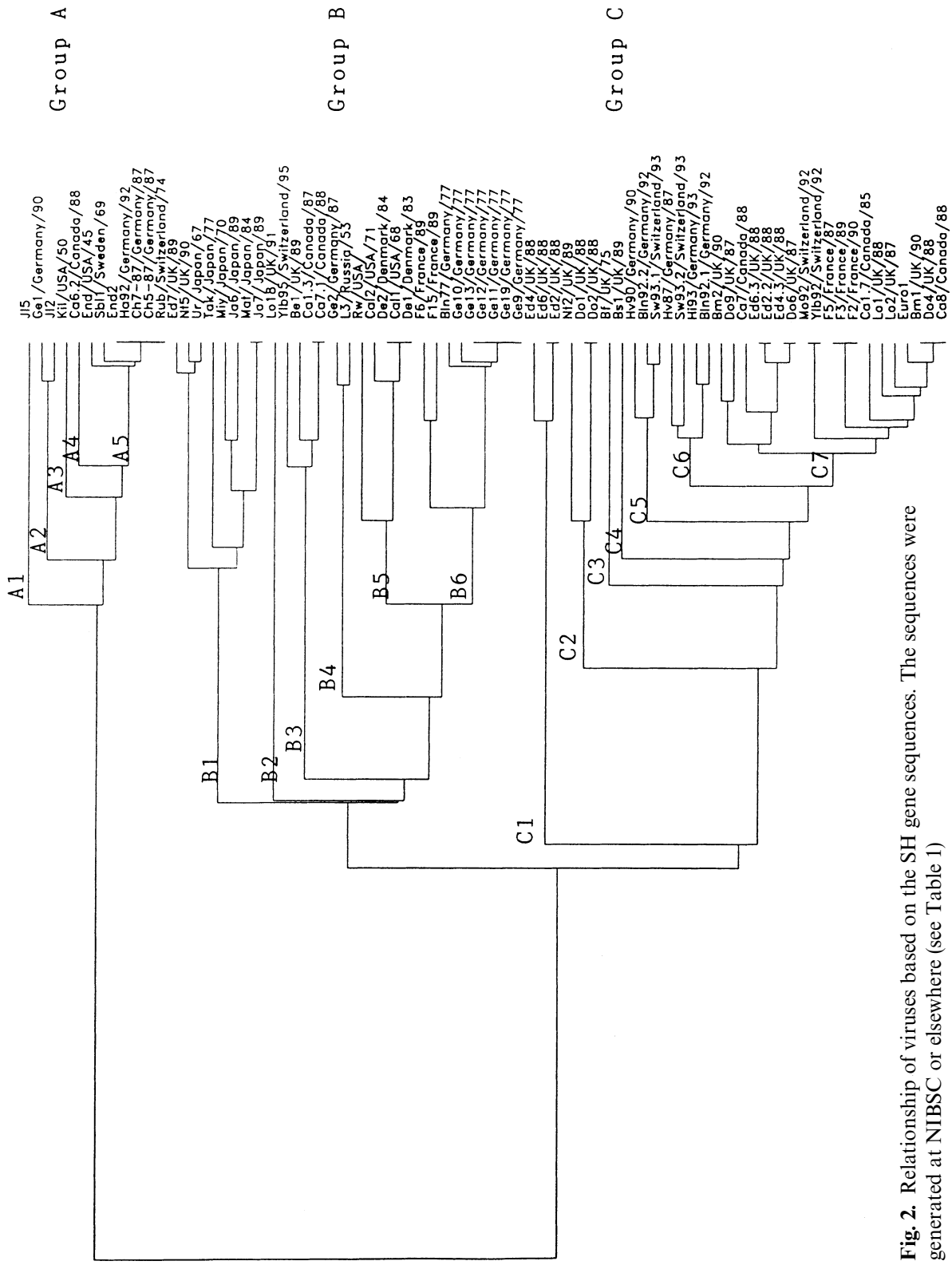

Fig. 2. Relationship of viruses based on the SH gene sequences. The sequences were generated at NIBSC or elsewhere (see Table 1)

and Ed4 isolates were returned to NIBSC and sequenced again, they had the same sequence as Ed2.2/UK/88 and Ed4.3/UK/88 respectively. The origin of the Ed2, Ed4 and Ed6 viruses whose sequence is published is therefore not clear, but they form a highly distinct subgroup within group C.

Group A included JL2 and JL5, the two component strains of the Jeryl Lynn vaccine, which originated in the USA, one isolate from a human recipient (Gel/Germany/90) and one from a monkey (Ca6.2/Canada/88) given the Jeryl Lynn vaccine. The Enders strain isolated in the USA in 1945 also fell into this group, although the sequence determined in the current study (End2) differed by one nucleotide (position 280, A → G) from the published [19]. As previously reported the Enders strain is very closely related to the Rubini vaccine strain [24] although the Rubini vaccine strain originated from a case of mumps in Switzerland in 1974. It seems possible that the Rubini vaccine is derived from the laboratory Enders strain although the passage history makes it difficult to see how this could have occurred (R. Glück, pers. comm.). Other viruses in group A included the well established laboratory strains SBL1 from Sweden and Kilham from the USA with three isolates from Germany, which were identical to End2 and Rubini.

Group B included two geographically well defined subgroups, namely B1, which comprised all published Japanese isolates and isolates Ed7/UK/89 and Nt5/UK/90, from recipients of the Japanese derived Urabe vaccine strain, and B6, which was composed of isolates from France and Germany. The remainder of group B was a mixture of North American and European strains.

The function of the SH gene is unknown, and a translation product has not been identified in vitro. The proposed coding region begins at position 51 and finishes at position 224, and this open reading frame was conserved in all isolates except Nt5/UK/90, obtained from a recipient of the Urabe vaccine, in which a point mutation in the second base of the initiation codon converted the AUG to AAG. The nucleotide sequence was obtained from multiple M13 clones and by direct PCR sequencing of a separate PCR preparation, and was otherwise identical to that of Urabe. The virus grew to titres comparable to other isolates, suggesting that the ORF was not necessary for growth in vitro.

Comparison of the large numbers of sequences now available showed that the first fifty nucleotides of the SH gene preceding the initiation codon were very highly conserved with at most two differences (i.e. 4%) from the consensus sequence (see sequence alignment acc. no. DS 26771). In contrast differences from the consensus sequence in the coding region from nucleotides 51 to 224 ranged from 4 to 23 base positions, or 2.3 to 13.4%, and in the 3′ non coding region from nucleotides 225 to 316 from 2 to 25 base positions or 2.2 to 27.2%. A high proportion of the differences in the coding region results in amino acid changes as reflected in their distribution within codons. Point mutations in the coding region relative to the consensus sequence occurred at 67 positions, of which 20 (29.9%) were in the first base, 19 (28.4%) in the second and 28 (41.8%) in the third. In contrast in the M gene 16.1% of variation from the consensus sequence occurred in the first base, 3.5% in the second and 80.5% in the third base. The

Table 2. Actual and percentage distribution of point mutations in the coding regions of the SH, M and HN genes

Gene	Coding region	First base	Second base	Third base	Total	No. of isolates compared
SH	171 nts (57 a.a)	20 (29.85%)	19 (28.36%)	28 (41.79%)	67	34 (acc. no. DS 26771)
M	1125 nts (375 a.a)	14 (16.10%)	3 (3.45%)	70 (80.46%)	87	7 [3]
HN	1746 nts (582 a.a)	35 (16.99%)	26 (12.62%)	145 (70.39%)	206	9 [24]

distribution of variation in the HN gene were 17.0, 12.6 and 70.4% at the first, second and third positions respectively, more closely resembling that found in the M gene than in the SH gene. These findings are summarised in Table 2.

Discussion

Limited genomic sequencing has proved to be of great value in the molecular epidemiology of a number of viruses, both in identifying strains as vaccine or wild type, and in studies of the circulation of viruses. The SH gene has been found to be a useful region of the mumps genome to study for the identification of strains because of its relatively high variability, although hitherto the relationships established are the same irrespective of the area examined.

The results reported here further extend and confirm previous reports based on sequencing of the SH and other genes of mumps virus. As before, the isolates form three major clusters, designated A, B and C, such that B and C are more closely related to each other than either is to A. Subclusters could be identified within the major clusters, some of which such as subclusters B1 and B6 were made up exclusively of isolates from defined geographical areas.

Other subclusters were not geographically defined. For example, subcluster B5 included isolate RW, a laboratory strain from America, Cal1/USA/68 and Ca12/USA/71 from California and isolates from Denmark (De1/Denmark/83 and De2/Denmark/84). It is conceivable that there are epidemiological links between the isolates, if for example De1/Denmark/83 and De2/Denmark/84 were imported from America, but this is entirely speculative.

The strains in cluster A include JL2 and JL5, the components of the Jeryl Lynn vaccine, Ge1/Germany/90 and Ca6.2/Canada/88 isolates respectively from a human and a primate given the Jeryl Lynn vaccine, three commonly used laboratory strains, namely Kilham, SBL-1 and Enders, and three isolates from mumps cases in Germany and the Rubini vaccine strain. The last four sequences were identical to that of Enders as determined here (End2), and identical to the sequence of the Rubini strain independently determined in our laboratory and elsewhere [14]. The identity of the Rubini and Enders sequences in a highly variable portion of the genome raises the possibility of contamination at some stage in the development of the Rubini strain.

Other examples of possible cross-contamination at some unknown point include the presence of two distinct strains in the Jeryl Lynn vaccine, although it

is conceivable that the original patient was infected with two strains, the anomalous position of Ed2, Ed4 and Ed6 compared to strains Ed2.2/UK/88, Ed4.3/UK/88 and Ed6.3/UK/88, which are closely related in terms of passage history and the isolate designated Euro 1, which emerged as a contaminant of an Enders preparation grown at NIBSC. One of the objectives of the work described here was to have a panel of mumps isolates of known molecular genetic relatedness to identify sources of possible future contaminants. In this regard, Euro 1 is closely related to but not identical with Bm1/UK/90, Do4/UK/88 and Ca8/Canada/88, all of which were grown in the laboratory at some time. It must be emphasised that even if the development of the Rubini and Jeryl Lynn vaccine strains did involve contamination with other strains, which is not proven, their safety and efficacy is well established by clinical usage. It would however suggest that contamination is a hazard even under the most careful conditions of passage.

The function of the SH gene is unknown, and its product has never been identified. One isolate, Nt5/UK/90, had a mutation in the putative initiation codon of the SH ORF, implying that a functional ORF is not necessary for growth in vitro. Moreover a high number of the nucleotide differences between strains resulted in predicted coding changes when compared to genes known to be translated into proteins. Fifty-eight per cent of differences were in the first or second base of the codon of the SH gene ORF, compared to 20% for the M gene and 30% for the HN gene. This suggests either that the SH gene ORF is not translated into protein, or that the protein is able to accommodate a high frequency of substitutions without loss of function.

In conclusion the data reported here extend previous studies of the molecular relationship between mumps isolates, confirming the existence of three major clusters encompassing geographically related subclusters. They also suggest the need for great care to be exercised to prevent laboratory contamination, which is not uncommon where viruses grow relatively poorly from clinical isolates, and that translation of the SH gene ORF is perhaps not necessary for virus growth in culture.

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