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SHORT PAPER

Characterization of a new genotype of measles virus detected in China and England

L. JIN[1,*] Y. J. SUN[2], L. GE[3] AND D. W. G. BROWN[1]

[1] *Enteric and Respiratory virus Laboratory, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT, UK*
[2] *Anti-Epidemic Station of Liaoning Province, Shenyang, P.R. China 110005*
[3] *Dalian Anti-Epidemic Station, Dalian, P.R. China 116021*

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SUMMARY

We report the co-circulation of a new lineage of measles virus (MV) and an Edmonston-like (Ed-like) genotype of MV in China during 1995–7. Sequence analysis of 25 strains was performed on a 282 nucleotides (nt) region of the nucleoprotein (N) gene, a 450-nt region of the haemagglutinin (H) gene and a 152-nt region of the matrix (M) gene by direct sequencing of RT-PCR amplicons obtained from clinical specimens. The entire H gene was sequenced from two strains. The results showed that 24/25 Chinese strains belonged to a new genogroup and were distinct from the vaccine strains used in China and the UK, and also from MV strains previously described in Europe, Africa and the USA. The remaining strain was Ed-like. Two strains of the new genotype (IV) and one of the Ed-like genotype were also detected in the UK in 1996.

Widespread measles vaccination programmes have greatly reduced the global incidence of measles since their introduction in the 1960s. However, measles is still a significant public health problem in many developing countries with measles infection causing nearly one million deaths each year.

Although measles virus (MV) is considered to be serologically monotypic, measles infections due to the wild-type virus can be distinguished from vaccine-associated cases, and the wild-type viruses can be classified into several genotypes which may have had a geographical origin [1–5]. The development of RT-PCR and sequencing methods has provided the possibility of molecular characterization of MV directly from clinical specimens by sequencing PCR amplicons [5, 6].

We have previously reported that at least three genotypes of MV were found cocirculating in the UK during 1992–5 and strains belonging to these three distinct lineages of MV continue to be identified [5]. Also new strains with distinctive sequences have been detected in sporadic cases in the UK, which we were unable to link directly to importation [7].

* Author for correspondence.

Identification of Chinese measles virus by RT-PCR

A small MV outbreak that occurred in Dalian, a city in Liaoning Province of China in 1995 was investigated. Subsequently, 25 MV strains from 6 outbreaks and 15 sporadic cases were identified in 5 cities of Liaoning Province. Two of MV strain were detected from patients who had typical clinical symptoms whilst travelling from Henan Province and Hebei Province (rural areas) to Liaoning. Both patients reported that MV outbreaks were occurring in their hometowns. Therefore, we named the strains as HN3

Table 1. *Source of measles virus strains in the study*

MV strain	Age	Location	Date onset*	Sample	Epidemiology characterization	IgM	Gene analysed†	Genotype
DL1	17	Dalian/China	6/1995	Throat swab	Outbreak 1	Pos.	h, m, n	IV
DL2	20	Dalian/China	6/1995	Throat swab	Outbreak 1	Pos.	m, n	IV
DL1	25	Dalian/China	4/1997	Throat swab	Sporadic	Pos.	h, m	IV
DL3	36	Dalian/China	5/1997	Throat swab	Sporadic	Pos.	h, m	IV
DL6	26	Dalian/China	3/1997	Throat swab	Sporadic	Pos.	m, n	IV
DL7	27	Dalian/China	3/1997	Throat swab	Sporadic	Pos.	m, n	IV
HN3	3	Henan/China	2/1997	Urine	Outbreak 2	Pos.	h, m	IV
SY2	9	Shenyang/China	2/1997	Saliva	Sporadic	Pos.	h, n	Ed-like
SY4	20	Shenyang/China	3/1997	Saliva, serum	Sporadic	Pos.	h	IV
SY5	23	Shenyang/China	3/1997	Saliva, urine	Sporadic	Pos.	h, m	IV
SY10	21	Shenyang/China	3/1997	Saliva, urine	Sporadic	Pos.	h, m, n	IV
SY11	20	Shenyang/China	3/1997	Saliva, urine	Sporadic	Neg.	h	IV
SY12	20	Shenyang/China	3/1997	Saliva, urine	Sporadic	Pos.	h, m	IV
SY13	20	Shenyang/China	3/1997	Saliva, urine	Sporadic	Neg.	H, m, n	IV
SY16	30	Shenyang/China	4/1997	Saliva, urine	Sporadic	Pos.	h, m	IV
SY17	28	Shenyang/China	4/1997	Saliva, urine	Sporadic	Pos.	h, m	IV
SY19	20	Shenyang/China	4/1997	Saliva, urine	Sporadic	Pos.	h	IV
SY37	16	Shenyang/China	6/1997	Saliva, urine	Sporadic	n.d.	n	IV
HB38	18	Hebei/China	5/1997	Saliva	Outbreak 3	Pos.	m	IV
HLD30	10	Huludao/China	3/1997	Saliva, urine	Outbreak 4	Pos.	n	IV
HLD32	11	Huludao/China	3/1997	Saliva	Outbreak 4	Pos.		IV
HLD36	7	Huludao/China	3/1997	Saliva, urine	Outbreak 4	n.d.	h	IV
PJ51	28	Panjin/China	5/1996	Saliva	Sporadic	Pos.	h	IV
PJ55	11	Panjin/China	5/1996	Saliva	Outbreak 5	Pos.	h, m	IV
FX23	21	Fuxin/China	4/1997	Saliva	Outbreak 6	Pos.	h	IV
UK80	35	Lincoln/UK	4/1996	Saliva	Sporadic	Pos.	h, m, n	Ed-like
UK168	1	London/UK	7/1996	NPA‡	Sporadic	n.d.	H, m, n	IV
UK178	4	London/UK	7/1996	Saliva	Sporadic	Pos.	m	IV

* Month/year.
† H, 1–1800 nt of the H gene; h, 450 nt of the H gene; m, 152 nt of the M gene; n, 282 nt of the N gene.
‡ Nasopharyngeal aspirate.
n.d., not done.

and HB38 (Table 1). Liaoning Province (area 270000 km² and population 40 million), is located at the Liaodong peninsula in the Northeast of China. Three MV cases (UK168/96, UK178/96 and UK80/96) identified in the UK were shown to be genetically related to Chinese strains.

The sources of clinical specimens are shown in Table 1. Anti-MV IgM was tested in saliva for UK specimens as described previously [5] and tested in Chinese serum specimens using an ELISA test (Beijing Institute of Biological Product Control, China). MV RNA was extracted from specimens including throat swab, saliva, urine and serum using the silica-guanidinum thiocyanate method [6]. The vaccine strain (ChgChina94, China) was analysed for comparison. RT-PCR was carried out as described previously [6]. In addition to the primer sets for the matrix (M) gene and for the nucleoprotein (N) gene [6], two sets of primers for a 450-nt fragment of the haemagglutinin (H) gene were also used in this study, primers Mh1 (5′-ACTACAATCAGAGGTCAA-TTC) and Mh2R (5′-AGCATGTCTCCATTCGCA-ACT) for the first round PCR, and primers Mh3 (5′-CAGAGGTCAATTCTCAAACA) and Mh4R (5′-CATTCGCAACTTGTCATCTG) for the nested PCR. PCR amplicons were cut and purified from the agarose gels for sequencing [6]. The primers for amplifying and sequencing of the entire H gene have been described previously [8]. Sequence was obtained from PCR amplicons including, 12 N genes, 20 H genes and 18 M genes from these 28 cases. The Taq dye deoxy-terminator cycle sequencing kit (Applied Biosystems) was used with the primers for the nested PCR in an ABI373A automatic DNA sequencer. Nucleotide and deduced amino-acid (aa) sequences were analysed with the SeqEd version 1.0.3 program

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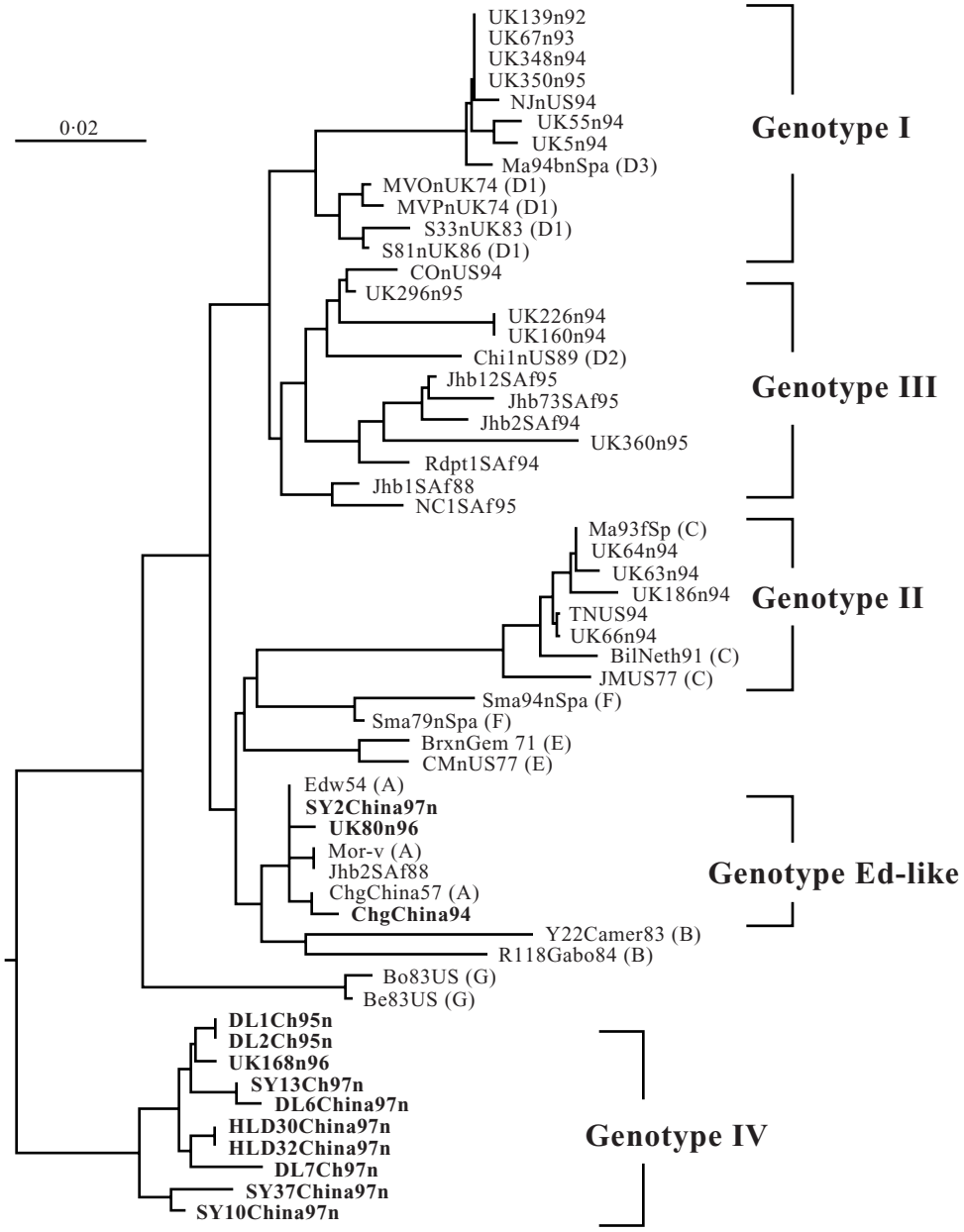

Fig. 1. Genetic relationships between new MV genotype (IV) and previously reported genotypes on the basis of the sequence nt1235–1516 of the N gene. Unrooted tree diagram was drawn using the Clustal routine of Megalign program in the DNASTAR package. Genotype designations from A to G (indicated in brackets) are as previously described [4]. Strains from the UK, from the USA and from S. Africa are refer to the published articles [2, 3, 6, 9]. Strains reported in this study are highlighted.

and Phylogenetic trees was drawn using Clustal of the Megalign program of the DNASTAR package.

Genetic characterization of a new MV genotype

The analysed genes and the results are shown in Table 1. As with earlier reports there was no evidence of recombination. The results demonstrated the reliability of examining three separate MV genes simultaneously and enabled MV strains to be characterized directly from clinical specimens. Twenty-four out of 25 specimens from China clustering into a new MV group which is different from previous reported genotypes [1–4, 6, 9]. Coincidentally, two strains detected in the UK were found to be closely related to this new group. We propose this group be classified as MV genotype IV until an international agreed nomenclature is established.

The C-terminal of the N protein is the most variable region of the MV genome and has been the most widely used for heterogeneity analysis of MV. Figure

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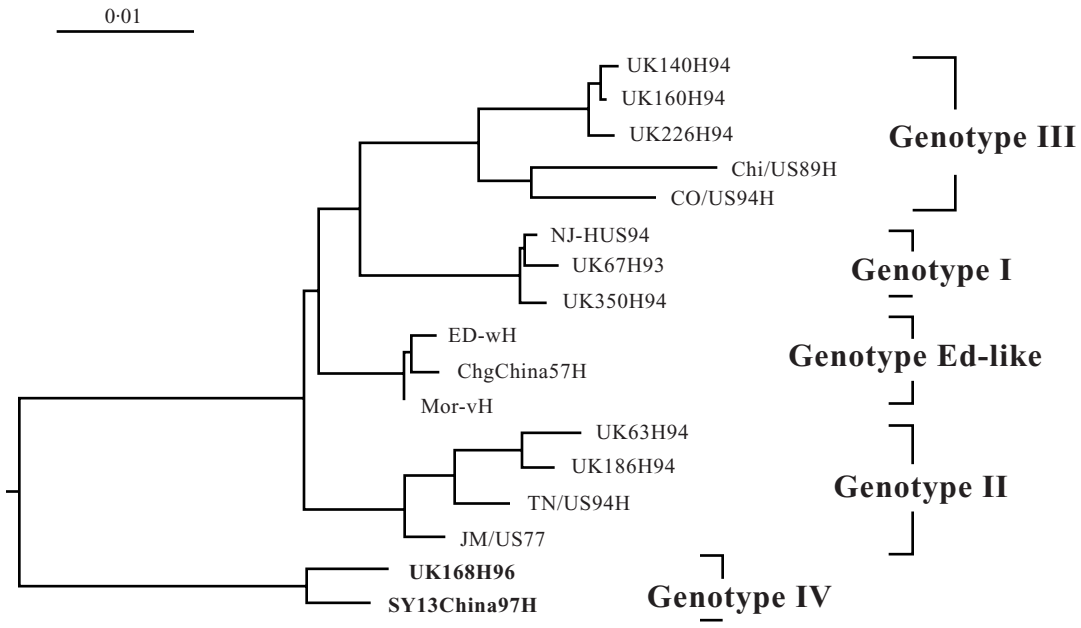

Fig. 2. Genetic relationships between MV genotype IV and previously reported genotypes. Unrooted tree diagram was drawn on the basis of the sequence nt1–1800 of the H gene using the Clustal routine of Megalign program in the DNASTAR package. Strains reported in this study are highlighted and strains reported previously refer to publication [8].

1 shows the genetic similarity between 13 of the MV strains including the vaccine strain (ChgChina94) currently used in China, and 44 previous reported strains characterised by analysing a 282-nt region (aa412-505) of the C-terminal N gene, which were detected in Europe, Africa and in the USA [1–4, 6, 8]. These previously reported strains were grouped as genotype I, II, III and Ed-like [5, 6] or genotypes from A to G (indicated in brackets in Fig. 1) by Rima and colleagues [4]. The divergence between these genotypes ranged between 2·5 and 1·06%. Nine Chinese strains and strain UK168n96 clustered into a new branch using Megalign program and were distinct from all published MV strains. The divergence of these strains when compared with previous reported strains or genotypes ranged between 6·7 and 13·1%, indicating that a new MV genotype has been identified circulating in China and in the UK. Four amino-acid (aa) substitutions were observed in all strains of this new genotype, S450→N/T, Y451→S, L473→P and S481→Y. In addition, the substitutions, D484→E occurred in strains DL1China95, DL2China95, DL6China97, SY13China97 and UK168n96; R438→K and R497→K in strains DL6China97 and SY13China97; G434→S in strains HLD30 and 32China97; G422→S in DL6China97 only; G445→R in DL7China97 and T469→A in UK168n96 only. There was no significant changes in the region aa457-476 which encodes a predicted B-cell epitope [10].

Complete sequence of the H gene of the new genotype strains

MV virus was also successfully cultured from two of the specimens, UK168n96 and SY13China97, using the B95a cell line as described previously [8]. The entire H gene of these two strains (accession numbers Y16098 and Y16096) were sequenced from the PCR amplicons of cell culture. Analysis of the nucleotide sequences of the coding regions (nt1-1800/aal-600) of the H gene also indicated that these two strain belonged to a new genetic group, genotype IV (Fig. 2). The divergence between genotype IV and other genotypes ranged from 4·7 to 6·7%, compared to a divergence of 1·4–4·3% between previously described genotypes. There were 18–28 aa differences from other genotype strains including several unique changes. Most of the important biological and immunological sites, such as all of the 13 cysteines were unchanged [11, 12]. However, one substitution from serine to asparagine at aa240 of the H gene were observed in both MV strains, which would destroy one of the five predicted *N*-linked glycosylation sites [11].

MV strains belonging to genotype IV were found to be diverse as has been found with genotype III strains [6], despite the narrow sampling range. The divergence within genotype IV strains characterized here based on the 282-nt region of the N gene was up to 3·5% (Fig. 1); up to 4% based on the 450-nt region of the

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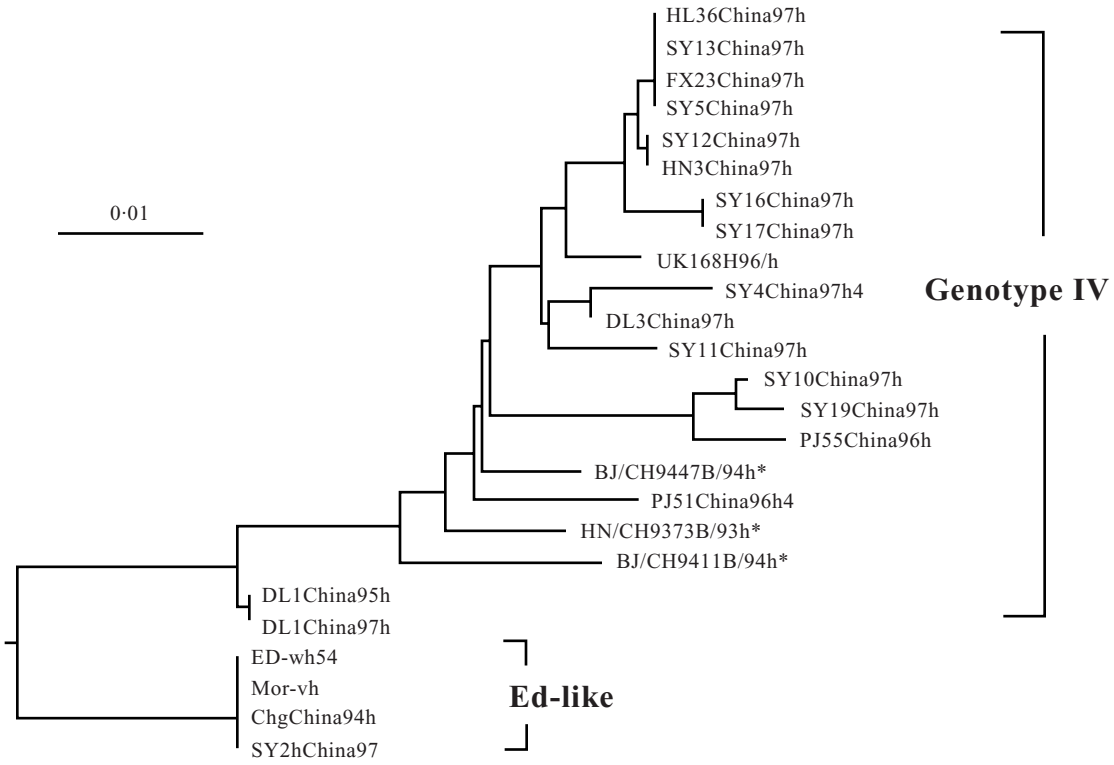

Fig. 3. Divergency between MV strains of genotype IV detected in China on the basis of the sequence nt628–1080 of the H gene. Unrooted tree diagram was drawn using the Clustal routine of Megalign program in the DNASTAR package. MV strain detected in the UK was boxed. The three strains with * were isolated in China during 1993–4 and the sequences were provided by Dr P. A. Rota (personal communication). Strains of the Ed-like group were used for comparison.

H gene (Fig. 3) and up to 2·6% based on the 152-nt region of the M gene (Fig. 4). There were three outbreaks in which strains from more than one case were characterized. Strains DL1 and DL2China95 from outbreak 1 had identical sequences in both the M and the N genes. Strains HLD30 and 32China97 from outbreak 4 showed identical sequences in the N gene. This is in agreement with our previous findings [6]. Identical sequences were also detected in strains identified between 1995 and 1997 (DL1China95 and DL1China97) based on the 450-nt of H gene, or from different locations, such as UK168/96 and SY13-China97 on the base of the 152-nt of M gene. However, the UK and Chinese strains were distinguishable from the N or H gene sequence. This suggests that genetic characterization based on multiple genes may be of value in epidemiological investigations.

We have shown that MV genotype IV has been circulating widely in China (Table 1). Two MV strains, UK168/96 and UK178/96, both belong to genotype IV and were detected in two unlinked British children who had neither travel histories nor foreign contacts prior to the illness. However, both cases were detected in July 1996 in London (Table 1), when many international travellers visit and the cases may be linked. We speculate that MV genotype IV might have been imported from China or other Asian countries, since these are the only genotype IV strains we have detected in the UK, despite extensive surveillance and the regular detection of genotypes I and II.

Ed-like strains also found in China and England

One of the 25 Chinese strains, SY2China97 from a sporadic case was found to be identical to the Ed strain based on both the C-terminal of the N gene (Fig. 1) and the 450-nt region of the H gene (Fig. 3), but 0·4% different from vaccine strain ChgChina94 based on the N-282 region (Fig. 1). Strain UK80/96, detected from a 35-year old Chinese student also clustered into the Ed-like genotype based either on the H gene or the N gene. Both strains SY2China97 and UK80/96 were detected from the patients with no recent vaccination history. Ed-like strain was also found in one of 14 isolates in China during 1993–1994 (Rota et al., personal communication). In addition, a small outbreak in 1993 caused by an Ed-like strain in

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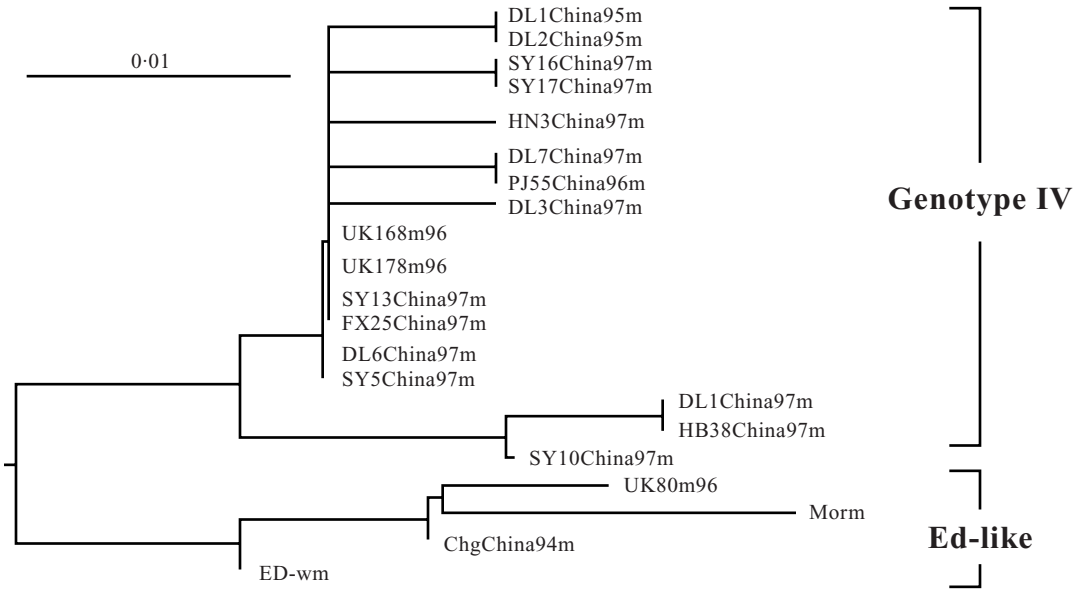

Fig. 4. Divergency between MV strains of genotype IV detected in China on the basis of the sequence nt130–281 of the M gene. Unrooted tree diagram was drawn using the Clustal routine of Megalign program in the DNASTAR package. MV strains detected in the UK was boxed. Strains of the Ed-like group were used for comparison.

the UK [13], suggests that the Ed-like wild strain is still circulating.

As has been described in the USA and in the UK [14, 15] the incidence and age of measles infection has risen in China since 1991. Forty-three percent of patients were more than 10 years old and adult cases have increased in epidemic areas [16]. Vaccination histories for most patients in this study were unknown except for DL7, HN3, SY2 and SY12 who were not vaccinated and SY11 who was vaccinated in 1978. A previous report from China [16] indicated that 36% of 11506 measles infections occurred in previously vaccinated individuals, the majority (92%) more than 7 years before illness. Two patients, SY11 and SY13 were negative for anti-MV IgM (Table 1), which may indicate a loss of vaccine-induced immunity, alternatively it may refer to the early collection of these samples (3 days after appearance of symptoms).

A high uptake of MMR and vaccination of all 5-16-year-olds in November 1994 in the UK has resulted in a low measles incidence in the UK [17]. However, MV may be continually reintroduced into the UK by international travellers. Monitoring the distribution of MV genotypes will provide valuable information about the transmission dynamics of MV in a community. As more extensive studies establish the geographic origin of MV strains a clearer picture of the geographic distribution will emerge. Continuing surveillance is likely to play an important role in future programmes to control measles.

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