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Characterization of measles virus strains causing SSPE: A study of 11 cases

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Eleven subacute sclerosing panencephalitis (SSPE) cases diagnosed in the UK between 1965 and 2000 were investigated. The entire or partial matrix (M), hemagglutinin (H), and nucleoprotein (N) genes of measles virus (MV) were sequenced following direct RT-PCR amplification from brain tissues. All the M genes showed the characteristic biased hypermutations and a premature termination codon was detected in 5/11 M sequences. Based on the more highly conserved H and N genes observed in persistent MV studies, phylogenetic analysis showed that two of three strains from patients likely to have acquired infection in the 1950s were related to clade C (WHO designation) and one appears to be a novel genotype. Three strains from patients infected in the 1960s and 1970s were clearly related to a MV strain isolated in 1974 belonging to genotype D1. Four strains from patients infected in the 1980s clustered with genotype D7 strains. One sequence from a patient infected in 1990s was identified as genotype D6. No vaccine strains were detected although five of these patients had been previously immunized. The sequence data obtained from these historic strains do not support the view that vaccine strains are associated with SSPE and provide valuable information for further studies of MV epidemiology, evolution, and pathogenesis in SSPE. *Journal of NeuroVirology* (2002) **8,** 335–344.

Keywords: measles virus; SSPE; genotyping

Introduction

Subacute sclerosing panencephalitis (SSPE) is a rare complication of measles infection and differs from acute measles encephalitis that appears during or after acute measles infection probably as a result of virus-induced autoimmune reactions, SSPE, and measles inclusion body encephalitis (MIBE) resulting from a persistent infection in neural cells (Griffin, 2001). SSPE develops in approximately four per 100,000 cases of measles. It occurs on average of 8 years after the initial infection and the main risk factor is acquiring infection at or before the age of 1 year (Miller *et al*, 1992).

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Measles virus (MV) is a member of the *Morbillivirus* genus in the Paramyxoviridae family, and it is serologically monotypic. MV genome is composed of a negative sense, nonsegmented ssRNA of 15,894 in length, which encodes six major structural proteins. Two surface glycoproteins, hemagglutinin (H) and fusion (F), induce neutralizing antibody responses. The H protein mediates attachment to susceptible host cells, interacts with the cellular receptors, CD46 and/or CDw150 (Manchester *et al*, 2000; Tatsuo *et al*, 2000) to allow MV entry, and sometimes triggers CD46 downregulation (Krantic *et al*, 1995). The matrix (M) protein is a membrane-associated protein and plays a key role in the assembly of virions (Udem and Cook, 1984) and controlling cell-to-cell fusion (Cathomen *et al*, 1998a). The M protein conformation is important for interaction with the viral nucleocapsid (Hirano *et al*, 1993). The nucleocapsid (N) protein, the phospho (P) protein, and the large (L) protein are components of the viral nucleocapsid and are essential for the MV replication and transcription apparatus (Sidhu *et al*, 1995; Griffin, 2001). Although MV is considered as an antigenically monotypic

Table 1 Characteristics of SSPE patients and measles strains analysed in the study

SSPE strain	*Patient location*	*Year of birth, (gender)*	*Year of MV infection/sample year*	*Age at SSPE diagnosis*	*Year of vaccination*	*Genotype*	*Gene sequenced***
UK83/56	Belfast	1953 (M)	1956/1965	12	No	A?	M, H*, N
UK85/56	Belfast	1950 (M)	1956/1965	15	No	C1	M, H*, N
UK88/55	Belfast	1948 (M)	1955/1965	17	No	C1	M, H*, n
UK86/60s	Belfast	1966 (F)	1960s/1974	8	No	D1	M, H*, n
UK87/69	Belfast	1967 (M)	1969/1974	7	No	D1	M, H*, n
UK157/80	Bristol	1979 (F)	1980/2000	21	No	D1	M, h
UK44/80s	London	1981 (M)	1980s/1997	15	1994	D7	M, H*, n
UK111/80s	Nottingham	1982 (F)	1980s/1998	15	1994	D7	M, H*, N
UK98/80s	Nottingham	1985 (M)	1980s/1999	10	1988, 1989	D7	M, H, N
UK99/80s	Cardiff	1984 (F)	1980s/1999	14	1995	D7	M, h, n
UK125/90s	Glasgow	1993 (M)	1990s/1999	6	1994, 1997	D6	M, H, N

*Data published in previous studies (Woelk *et al*, 2001, 2002).
**Upper-case letters represent the entire gene and lower-case letters represent a partial region of genes.

virus, significant genetic diversity exists. Eight clades (A–H) including 21 genotypes of MV have been recently assigned by WHO based on sequence differences in the N and the H genes (WHO, 2001). In contrast with the typical wild (acute) MV strains, aberrant M protein due to major mutations in the M gene is believed to be the hallmark of persistent MV infection in SSPE patients (Cattaneo *et al*, 1989; Billeter and Cattaneo, 1991). The main features include the defective M protein, the truncation and distortion of the cytoplasmic domain of the F protein, and the reduced hemadsorption and inefficient membrane transport due to hypermutations in the M, F, and H genes, which may interfere with virus budding (Schmid *et al*, 1992; Schneider-Schaulies *et al*, 1995; Schneider-Schaulies and ter Meulen, 1999).

The incidence of SSPE has fallen in direct association with falling measles cases due to rising coverage of measles vaccination (Miller, 2002). However, there has been extensive debate in recent years about potential adverse events following MMR vaccination such as Crohn's disease and SSPE. Currently there is no scientific support for these links but it does highlight the importance of characterizing measles strains in conditions such as SSPE and encephalitis in patients with a history of measles or MMR vaccination. In this study MV strains were characterized directly from postmortem or biopsy specimens of SSPE patients using a nested RT-PCR and sequencing. Our aims were to investigate if the measles strain used in the vaccine introduced in 1968 in the UK could cause SSPE cases, to determine if any measles genotypes are strongly associated with SSPE, and to identify measles virus diversity by comparing the historic strains obtained from SSPE patients with current wild-type strains, which may contribute to our understanding of MV evolution.

Results

Measles genome was detected by RT-PCR in all brain samples available from the 11 patients. The MV genes amplified from brain tissue specimens included the entire M gene (1,008 nt) for all 11 cases, the entire H (1,854 nt) for strains UK98 and UK125, and a variable region (510 nt) of the H for strain UK157 and UK99. The entire N gene (1578 nt) was sequenced for strains UK83, UK85, UK111, UK98, and UK125 and the C-terminal N gene (282 nt) for the remaining cases except strain UK157 due to insufficient specimen (Table 1). The complete M and H genes of strain Loss88 were also sequenced. None of the nine brain specimens obtained from non-SSPE adults were positive by RT-PCR assays.

Sequence variations in the M, H, and N genes
The entire M gene was sequenced for these 11 SSPE patients. When compared with the consensus sequence, the average percentage of transition U to C (as read in the plus-strand polarity) for these 11 SSPE strains was 73.3%, at least six times higher than other substitutions (data not shown); whereas it was 22% for the acute strains based on five of the sequences (Table 2). The Ts/Tv was 30.45 for these 11 SSPE strains (Table 2). Newly generated termination codes were detected in 5 of 11 M genes. These termination codes were randomly located at amino acid (aa) 88 of UK83, aa296 of UK85, aa22 of UK86, aa12 of UK157, and aa306 of UK111. The start code (Methionine) of

Table 2 Comparison of nucleotide substitution frequencies in the M, H, and N genes between MV strains obtained from acute cases and SSPE cases

Gene (nt)	*Data set*	*Taxa*	*U to C change (%)*	*Ts/Tv*	*Average of total substitutions (%)*
M	SSPE	11	73.3	30.446	3.3*
(1008)	Acute	5	22.0	7.718	1.2
H	SSPE	9	33.2	6.524	1.5
(1854)	Acute	5	22.6	5.123	1.3
N	SSPE	5	25.7	6.375	1.9
(1578)	Acute	5	25.7	5.983	1.8

*Number of the SSPE group is too small for value statistical analysis.

Table 3 Amino acid changes which may affect sites currently recognized as having important biological and immunological functions

		SSPE Strain (UK)										
		1950s			*1960s*		*1970s*	*1980s*				*1990s*
Gene (codon)	*Cons.*	*83*	*85*	*88*	*86*	*87*	*157*	*44*	*111*	*98*	*99*	*125*
M (335)	Met1											Thr
	Trp12						Ter					
	Gln22				Ter							
	Glu88	ter										
	Trp296		ter									
	Gln306								ter			
H (617)	Arg7	Gln					—	Gln			—	
	Ile8			Thr		Thr	—				—	
	Phe11						—		Leu	Ser	—	
	Tyr12	His		His			—		His		—	His
	Leu30			Pro			—				—	
	Mel31		Thr				—				—	
	Ile50						—			Val	—	
	Arg62			Gln			—			Gln	—	Gln
	Mel201			Thr			—				—	
	Leu206		Ser									
	Asp207					Glu						
	Ser247											Pro
	Gln248							Glu				
	Leu249											Pro
	Gly302							Lys	Lys	Arg	Lys	
	Glu303	Lys										
	Val345											Mel
	Ile346						Val					
	Arg348							Gly	Gly		Gly	
	Val357			Phe			Ile					
	Asp416						—	Asn	Asn	Asn	—	
	Leu423	Pro					—				—	
	Ile425						—			Val	—	
	Ala463		Pro				—				—	
	Ile473				Val		—				—	
	Phe476	Leu					—				—	
	Lys560		Arg			Arg	—	Arg	Arg		—	
N (525)	Leu58	Ser		—	—	—	—	—			—	
	Asp63	Asn		—	—	—	—	—			—	
	Asp239			—	—	—	—	—		Asn	—	
	Ser132	Leu	Pro	—	—	—	—	—			—	
	Asp135			—	—	—	—	—		Asn	—	
	Ser139	Asn	Arg	—	—	—	—	—			—	
	Phe144			—	—	—	—	—	Ser		—	Ser
	Ser460						—			Gly		
	Asp461				Glu		—					
	Pro468						—				Ser	
	Thr469	Ile					—					
	Ser470	Gly	Asp	Asp			—	Arg				
	Pro472	Ser					—					
	Leu473						—		Pro	Pro		
	Asp523	Asn	Asn	—	—	—	—	—	Asn	Asn	—	Asn

Blank, remains no change.
—, Not tested.

the M gene was altered to Threonine due to a T-C change in UK125 (Table 3).

The complete H genes for strains UK98 and UK125 and the 7 previous published strains (Woelk *et al*, 2001, 2002) were analyzed for the substitution frequencies. In contrast to the M gene, sequence replacements of U with C showed no significant differences between SSPE strains and acute MV strains, 33.2% and 22.6%, respectively (Table 2). The Ts/Tv was 6.524 for the SSPE strains (Table 2).

The substitution frequencies of the N gene were compared between the five SSPE strains and the five acute MV strains. Replacements of U with C data in the plus strain accounted for 25.7% of all mutations in both the SSPE strains and acute strains obtained from typical measles cases (Table 2). The Ts/Tv ratio was 6.375 for the five SSPE strains and 5.983 for the five acute strains (Table 2).

In addition to the newly generated termination codes in some strains of the M gene, nonsynonymous

(d_N) substitutions leading to codon changes, which may be involved with the sites currently recognised as having important biological and immunological functions were listed in Table 3. These include the previously identified B-cell epitopes (BC), T-cell (TC), T-helper, and cytotoxic T-lymphocyte (CTL) epitopes in the H and N genes.

Genotypes and evolutions of SSPE strains

The average total substitutions of the SSPE strains were 3.3%, 1.5%, and 1.9% for the M, H, and N, respectively. The average transition from U to C and the Ts/Tv ratio was similar for the H and N genes when comparing the SSPE group to the acute MV group (Table 2). Therefore, the H and N sequences of these persisting MV strains were reliable for performing phylogenetic analysis.

The C-terminal N gene of the 10 SSPE strains (no data available for UK157) and the H gene sequences of these 11 SSPE strains were compared with the 25 reference strains of the WHO designation for MV genotypes (WHO, 2001). The phylogenetic trees drawn based either on the H (Figure 1A) or the N gene (Figure 1B) showed that these SSPE strains clustered into four clades. UK85 and UK88 were related to genotype C1, UK86, UK87, and UK157 belonged to genotype D1, UK44, 98, 99, and 111 related to genotype D7, although only 510 nt were sequenced for UK99. UK125 was related to genotype D6. These four clusters correlated well with the presumed periods of the patients' primary infection, in the 1950s, 1960s, 1980s, and 1990s, respectively. UK83 appears to be unique and cannot be clearly related to any genotype. The divergences between strain UK83 and all genotypes ranged from 1.9% (genotype A) to 4.8% (genotype G3 or H1) based on the H gene and from 1.6% (genotype A) to 8.0% (genotype D7) based on the N gene. None of these SSPE strains was close to the vaccine strain (Mor) or the Loss strain that was used throughout the study as positive control.

Discussion

Most nucleotide substitutions are presumably due to errors of the polymerase, and the rate of mutation (e.g., U to C) would be expected to be equal. The biased hypermutations due to the activity of a cellular dsRNA-dependent unwinding/modifying enzyme (an enzymatic activity, termed unwindase) has been known as a phenomenon of the M gene from SSPE patients (Billeter and Cattaneo, 1991; Billeter *et al*, 1994; Ayata *et al*, 1998). In this study, the average percentage of transition U to C (as read in the plus-strand polarity) of 11 SSPE persisting MV strains was 73.3% in the M gene, at least six times higher than other transitions occurred. This was also three times higher than the average percentage of transition U to C of the 5 acute MV strains. The Ts/Tv ratio was 30.446 for the M gene of the SSPE strains, while 7.718 for the 5 acute MV strains, which is similar to the previously reported data, 6.261 based on 20 taxa (Woelk *et al*, 2001).

In addition to the high transitions detected in the M gene of these persisting MV strains, premature termination codons were randomly detected in 5 of 11 SSPE cases, and the initial codon was altered to Thr in UK125, which would functionally inactivate the M protein. The M protein is essential for MV assembly (Billeter *et al*, 1994), for encoding the viral envelope glycoproteins (Wild and Buckland, 1995) and that deletion of M increases cell-to-cell fusion and decreases production of infectious virus (Cathomen *et al*, 1998a; Naim *et al*, 2000). The M protein is a key mediator for virus budding, and it regulates sorting of the glycoproteins to the apical cell surface and viral release (Naim *et al*, 2000). No premature stop codon or altered start codon was detected in five of these 11 SSPE strains and these M genes were randomly mutated and did not show any clear patterns. However, the mutations in the amino- and carboxy-terminal regions can alter the M protein conformation that could abrogate nucleocapsid-binding function (Hirano *et al*, 1993). The hypermutated M proteins therefore may be of great importance for SSPE pathogenesis.

Compared with the M sequences the H and N genes of SSPE strains were highly conserved. There were only small differences in the Ts/Tv ratio for the H and for the N genes between SSPE and acute groups (ranged from 5.123–6.524, Table 2), which is similar to the previously published data, 5.125 for the H gene based on 50 taxa (Woelk *et al*, 2001). The Ts/Tv ratio for the M gene of the acute group was 7.718, similar to the previously published data, 6.261 based on 20 taxa (Woelk *et al*, 2002), while 30.446 for the M gene of the SSPE group. The average total changes of the M gene of SSPE group were 2.7 times higher than that of the M gene of acute group, whereas there was little difference between these two groups in the H and N genes. Therefore, the H and N sequences of these persisting MV strains were reliable for genotyping with phylogenetic analysis.

The characteristic mutations for SSPE strains were not identified in the H and N genes, whereas the 11 strains were distributed into at least four clusters on the phylogenetic tree based on when the primary infection happened. Two of the three sequences from patients likely to have acquired infection in the 1950s were related to genotype C1. Two sequences from the patients infected in the 1960s were clearly related to genotype D1. Four sequences from patients infected in the 1980s were related to genotype D7. Interestingly, UK125, presumed primary infection in 1990s belonged to genotype D6, which was one of the predominant genotypes circulating in the UK in the 1990s (Jin *et al*, 1997). UK157 from a SSPE patient in Bristol (Table 1), who was primarily infected with MV in 1980, belonged to genotype D1, of which the reference strain Bris.UNK74 was originally isolated in Bristol.

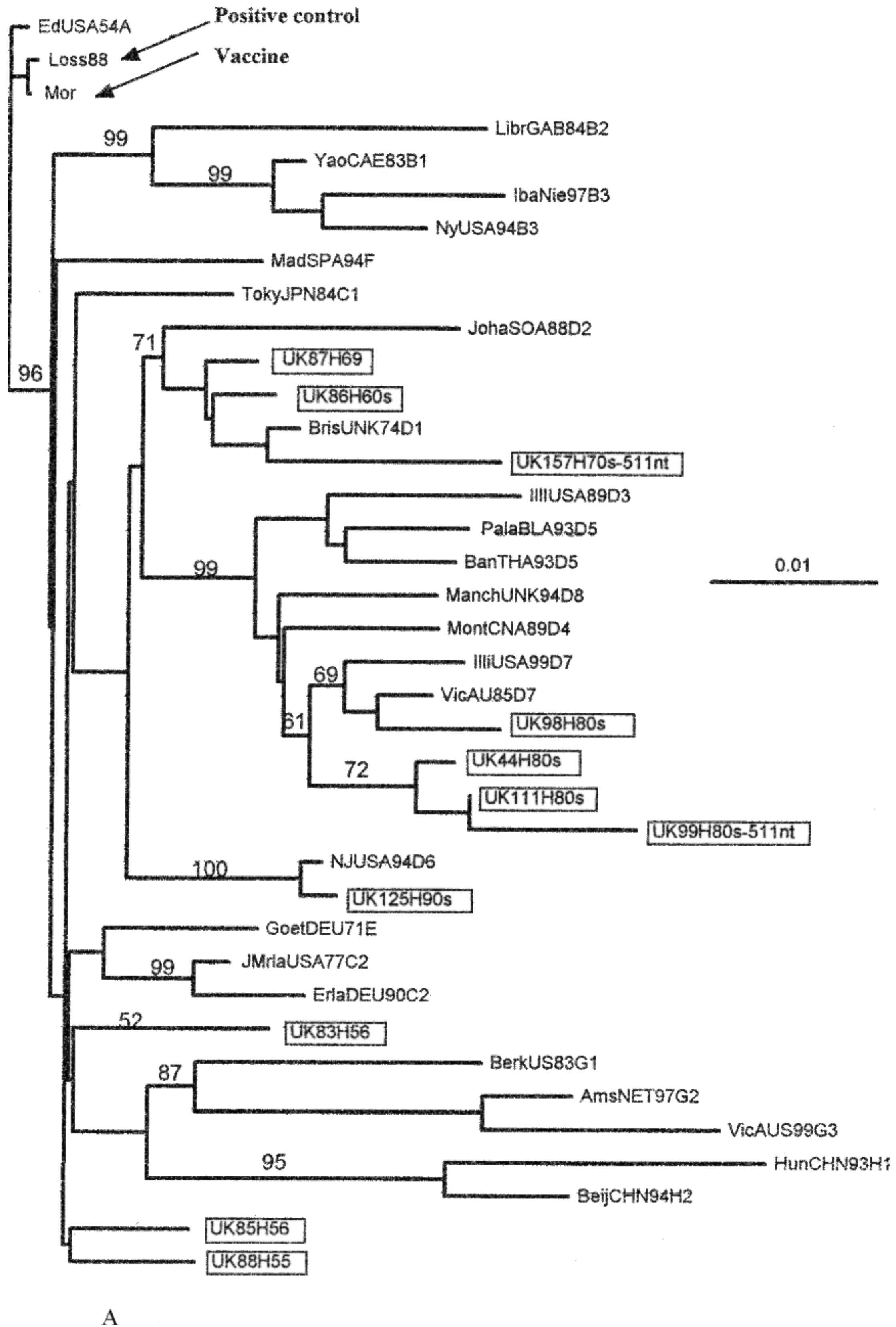

Figure 1 Genetic relationships between 11 SSPE (blocked) and the 25 reference strains with the designated WHO measles genotype are indicated at the end of each strain designation. The phylogenetic trees were drawn by bootstrap analysis (1000 times) using the neighbor-joining method (PAUP 4.0 Beta 2 package). (**A**) Phylogenetic analysis based on the entire H gene. (**B**) Analysis based on the C-terminus of the N gene (456 nt). *(Continued)*

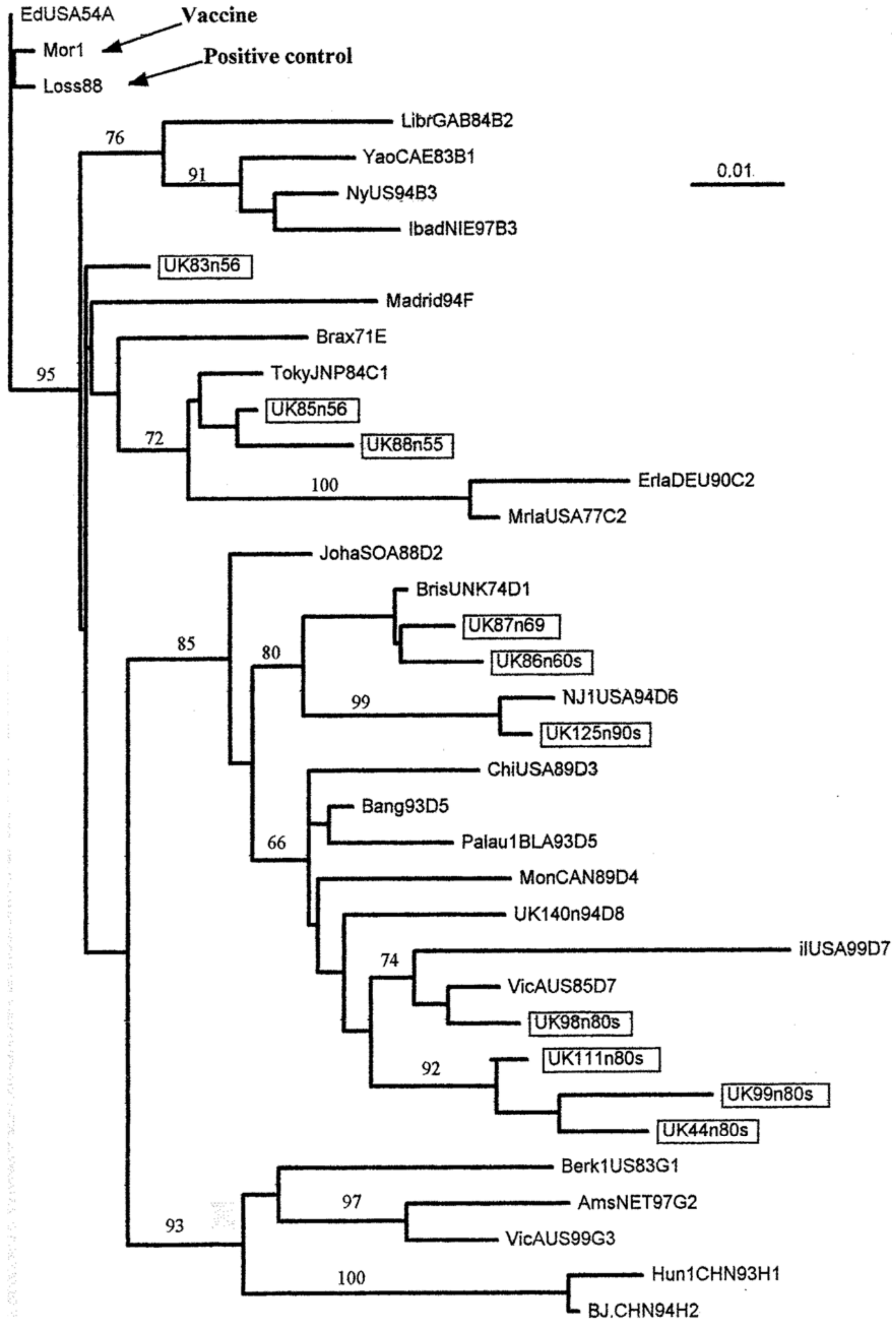

Figure 1 (Continued).

Although there was only a partial sequence of the H gene available (Figure 1A), it was most closely related (divergence at the lowest 1.6) to the D1 reference strain. The results suggest that these SSPE strains could be ancestors of current MV genotypes or strains, which may represent the predominant MV strains circulating in the 50s, the 60s to the 70s, the 80s, and the 90s, respectively, in the UK. The sequence data would be valuable in MV evolution studies. Similar findings supporting the view that SSPE sequences were related to wild-type measles viruses circulating years before the onset of SSPE have been described previously (Rima *et al*, 1997; Ayata *et al*, 1998; Vardas *et al*, 1999). UK83/56 appears to be unique on the phlyogenetic trees (Figure 1). However, the smallest divergence was 1.6 and 1.9 between UK83 and Ed/USA54, the reference strain of genotype A based on the H and the N genes, respectively, suggesting it might be an inactive historic strain belonging to genotype A.

Five of these SSPE patients had been vaccinated (Table 1) and none of these patients had a clear record of primary natural measles infection. However, none of the virus sequences was related to the vaccine strain (Figures 1A and 1B), suggesting these patients were exposed to wild-type MV prior to the vaccination and that the wild-type viruses were the cause of SSPE. The possible markers that may distinguish the vaccine and wild-type measles strains, such as aa positions 89 in M, and 211 and 546 in H were reported (Parks *et al*, 2002). Sequence data generated in this study showed that all belonged to the wild-type measles virus. In most cases vaccination was likely to have occurred after wild-type measles infection. There has been speculation that vaccination might activate a latent MV and lead to the development of SSPE (Wakefield, 1998). However, there is no evidence to support this suggestion and there is substantial information against it. In the UK, no increase in SSPE cases was seen following the 1994 Measles and Rubella National Campaign in which over 7 million school children were vaccinated (Miller, 2002). Two of the four female SSPE patients, UK157 and UK99, were 30 and 32 weeks' pregnant, respectively, when they were diagnosed as SSPE. It is not clear if there might be any links between hormonal changes during adolescence or pregnancy in young women and the development of SSPE. The factors that trigger the disease process remain unknown.

Immune responses to defective MV persistent in central nervous system have been postulated to play an important role in establishing SSPE process (see review by Schneider-Schaulies and ter Meulen, 1999). Analysis of aa sequence variations in the H and N genes revealed that there were significant changes (Table 3) affecting sites currently recognized as having important biological and immunological functions. In the H gene, these sites include potential B-cell epitopes at aa201-215, aa343-353, aa411-425, aa463-477, aa516-575 (Muller *et al*, 1993; Obeid *et al*, 1994), and a neutralizing domain at aa244-250, the antibodies of which prevent virus-cell fusion and protected against MV encephalitis in mice (Fournier *et al*, 1997), T-cell epitopes at aa423-437, aa443-457, aa473-487, aa543-557 (Obeid *et al*, 1993), and Cytotoxic T-lymphocyte (CTL) epitopes at aa29-37 and aa41-55 (Muller *et al*, 1993; Nanan *et al*, 1995). CTL responses to measles virus play an important role in recovery from infection. Random changes at epitopes, such as Leu423→Pro in UK83; Leu206→Ser and Ala463→Pro in UK85; Leu30→Pro in UK88; Gln248→Glu in UK44 and Leu249→Pro in UK125 (Table 3) may affect immune recognition or changes in structural domains that may result in dysfunctional proteins. Asp416→Asn, postulated to affect hemagglutination (Saito *et al*, 1995), was found in three SSPE strains in the 1980s. An important function of the glycoprotein tails, e.g., the amino-end of H protein, is to regulate cell fusion in addition to being involved in virus envelope assembly (Cathomen *et al*, 1998b). Alterations at the H tail (aa1-34, cytoplasmic domain) may affect virus induced cell fusion and envelope assembly. Interestingly, mutations in patterns of these SSPE strains were mostly located at the positive selective (adaptive evolution) sites (Woelk *et al*, 2001, 2002), and not related to genotypes. Mutations at aa12, 62, and 348 were (so far) only detected in SSPE strains, including the new data generated in this study, UK98 and UK125, and the results were in agreement with the previous report based on computer analysis (Woelk *et al*, 2002), suggesting that different selection pressures appear to be affecting the evolution of the envelope glycoproteins from acute and persistent cases of MV.

Mutations in the N gene were mostly located in the carboxy-terminal end, which were in patterns related to genotype classifications. Cell-mediated immunity has a critical role in the control of MV infection. T-cell epitopes identified in the N gene were concentrated in a few sites at the amino-terminal end (Nanan *et al*, 1995; Marttila *et al*, 1999). Mutation Asp239→Asn of the UK98 located at T-helper epitope aa221-240 may affect the long term T-help cell memory. Some CTL epitopes were identified in the N protein (Nanan *et al*, 1995; Schadeck *et al*, 1999); however, only two mutations were detected in UK83 at CTL epitope aa51-65. Random changes in these SSPE strains (Table 3) were found at the antigenic sites, aa122-150, aa457-476, and aa519-525 reported in previous studies (Buckland *et al*, 1989; Komase *et al*, 1990). The potential role of the mutations in the pathogeneses of SSPE needs to be investigated further.

Sequencing different genes—M, H, and N—separately has eliminated cross-contamination. The sequence data set of SSPE strains detected directly from clinical specimens without passage or cloning procedures provides valuable information for the

epidemiology, genetic evolution, and pathogenesis of measles infection.

Materials and methods

Patients and specimens

Fresh frozen brain tissue or biopsy specimens obtained from 11 patients were investigated. In all these cases, diagnosis was confirmed by clinically and by EEG or by measles antibody tests in CSF. The clinical information for some of these cases was published previously (Connolly *et al*, 1967, 1968; Jayawant *et al*, 2000). Table 1 summarizes the clinical information of 11 SSPE patients investigated in this study. Five patients born since the 1970s were all immunized once or twice with measles vaccines. Two (UK157 and UK99) of the four female patients were 30 and 32 weeks' pregnant when they were first diagnosed as SSPE.

Two of the 11 cases in this study had more than one sample available, two samples from UK83/56 (frontal and temporal cortex), and five samples from UK98/80s (frontal, temporal, parietal, occipital, and cerebellum cortex). The temporal cortex was obtained from postmortem of UK88/55, the left parietal occipital and cerebral biopsies were obtained from UK157/80 and UK125/90s, respectively. The brain cortices from another six patients were unknown. All samples were fresh-stored at −70°C. Nine brain specimens obtained from non-SSPE adults were tested by RT-PCR assays as controls. A lab-adapted MV strain Loss88 (Sinitsyna *et al*, 1990) was used as positive control in this study.

RT-PCR for amplification of the M, H, and N genes

Specimens were homogenized and RNA was then extracted and purified using the silica-guanidinum isothiocyanate method as previous reported (Jin *et al*, 1996). The RNA was then reverse transcripted using random hexamers (Jin *et al*, 1996), followed by a nested PCR amplification of the entire H gene (Jin *et al*, 1998). Similar procedures were carried out for PCR amplification of the entire M, N, and F genes. Primers selected for the first round PCR of the M gene were M5′-1 (5′-cttaggagcaaagtgattgc) and M3′-1R (5′-ttctggctgtcattgtgagg) and for the nested PCR, M5′-2 (5′-tgattgcctcccaagttcca) and M3′-2R (5′-gtcgttttcgggcattgctg). Primers for amplifying the N gene were N5′ and N3′ for the first round PCR (Rota *et al*, 1994) and N5′-1 (5′-gaattcgagcaggattaggcatatcc) and N3′-1 (5′-tcgagcggcctctcgcacctagtcta) for the nested PCR.

Nucleotide sequence determination and analysis

DNA fragments were cut from agarose gels (2%) and purified using the Geneclean method (BIO 101 Inc, USA). Sequencing reactions were performed using the *Taq* DyeDeoxy terminator cycle sequencing kit in an ABI 377A automatic DNA sequencer (Applied Biosystems Ltd, Warrington, UK). The forward and reverse primers for the nested PCR and the internal primers constructed for the entire H and N genes were used for sequencing as described previously (Jin *et al*, 1998; Rota *et al*, 1994). For the entire M gene, in addition to the forward and reverse primers, M3987 (5′-aatgcagtggccttcaacct) and M2r (5′-aacaactatgtcaagctcag) were also used for the sequence determination.

Nucleotide and deduced amino acid (aa) sequences of the SSPE cases were analyzed using the Clustal V and the Megalign programs of the DNASTAR package, and compared with the consensus sequences. The consensus sequence of the M gene was constructed from 35 sequences, including 17 acute (wild-type), 2 vaccine, and 4 SSPE strains, which were downloaded from the GenBank, and these 11 SSPE strains and strain Loss88 characterized here. The consensus sequence of the H gene was constructed from 50 sequences from the Global data set (Woelk *et al*, 2001). The consensus sequence of the N gene was constructed from 23 sequences, including the 17 genotype reference strains of which the entire sequences are available (WHO, 2001), the Loss88 strain, and the five SSPE strains in this study.

Nucleotide substitution frequencies were calculated for the M, H, and N genes of SSPE cases and a group of MV strains from the acute MV group. The sequence data of acute MV strains were downloaded from GenBank. The accession numbers for the acute-M are X16565, U01980, U01983, and AB002688; for the acute-H are X16565, M81895, M81898, and AB045300; and for the acute-N are X16565, U01977, M8992, and AF280803. Strain Loss88 was also included in the acute group with the N sequence provided by Dr P Rota (CDC, USA). The M and H genes of Loss88 were sequenced in this study. Transition/Transversion ratio (Ts/Tv) was estimated using the maximum likelihood of the PAUP 4.0 software package. Phylogenetic trees of the entire H and the *C*-terminal N (456 nt) genes were drawn by bootstrap analysis (1000 times) using neighbor-joining of the PAUP 4.0 software package.

The sequence data generated in this study was submitted to GenBank and accession numbers are AF503521–AF503532 and AF504040–AF504054.

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