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Molecular characterization of measles virus strains causing subacute sclerosing panencephalitis in France in 1977 and 2007

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6 2 **panencephalitis in France in 1977 and 2007**
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3 47 **Abstract**
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8 49 Measles virus strains from two subacute sclerosing panencephalitis (SSPE) cases diagnosed in
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10 50 1977 (Laine strain) and in 2007 (Hoedts strain) were studied. Phylogenetic analysis based on C-
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12 51 terminal part of the nucleoprotein and the entire H gene showed that Hoedts strain, circulating in
13
14 52 France presumably in the 1980s, belonged to genotype C2. However, Laine strain, suspected to
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16 53 have circulated between 1940s and 1960s, could not be assigned to any known measles virus
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18 54 genotypes. Sequences analysis of the Laine strain suggested that it originated from a measles
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20 55 virus that may have circulating at the same period as the Edmonston strain. The analysis of the
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22 56 whole genome of both SSPE strains revealed biased hypermutations in M, F and H gene. Some
23
24 57 of these mutations like the L165P found in the M protein sequence of the Laine strain, the amino
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26 58 acid position 94, where a mutation M94V was found in the F protein sequence of the Hoedts
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28 59 strain are known to play an important role in the glycoprotein interaction and to impair the ability
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30 60 of measles virus strain to produce cell-free infectious viral particles. This is the first study on
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32 61 molecular characterization of the entire coding region of measles virus isolated from SSPE cases
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34 62 in France.
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70 Introduction

71 Subacute sclerosing panencephalitis (SSPE) is a fatal disease of the central nervous system that
72 generally develops 7 to 10 years after infection by the measles virus. However, even with the
73 elimination of measles, cases of SSPE may still occur 20 to 30 years later because of the skew of
74 the latency distribution. Despite the availability of efficient vaccines and widespread vaccination,
75 measles remains a major cause of child mortality worldwide. An estimated 164 000 people died
76 from measles in 2008 [WHO, 2009]. SSPE is caused by a persistent measles virus infection of
77 the brain. According to the WHO, the incidence of SSPE is approximately 4-11 cases per 100
78 000 cases of measles [WHO, 2006]. Clinical manifestations of SSPE include behavioral
79 abnormalities, cognitive decline, myoclonic jerks, seizure and abnormalities in vision [Garg,
80 2008; Mahadevan et al., 2008]. Death generally occurs 1 to 3 years after onset of symptoms. The
81 reason why measles virus persists in some individuals is unknown, but is likely to be host
82 related.

83 Measles virus belongs to the paramyxovirus family and is a member of the *Morbillivirus* genus.
84 It is an enveloped virus whose genome contains six genes that encodes for six structural proteins:
85 nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), fusion protein (F),
86 haemagglutinin (H) and large protein (L). The P gene also encodes several other proteins C, V,
87 and W. Sequence analysis of SSPE viruses indicate that they differ from wild-type viruses due to
88 the introduction of several mutations that mainly affect the matrix, haemagglutinin, nucleocapsid
89 and fusion genes [Ayata et al., 2007; Jiang et al., 2009]. These genetic mutations in SSPE virus
90 result in poor expression of envelope proteins. Consequently, the SSPE virus is able to maintain
91 a persistent infection in neuronal cells of the brain but is unable to produce transmissible
92 infectious viral particles [Oldstone et al., 2005].

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3 93 In France, a nationwide case-based mandatory reporting of measles cases was established in
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5 94 2005. The vaccination coverage was approximately 87% at 24 month of age in 2005 [Waku-
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8 95 Kouomou et al., 2010]. This is lower than the 95% requested to stop the circulation on measles
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10 96 virus in the population. As result, a number of measles outbreaks were reported in recent years
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12 97 [Waku-Kouomou et al., 2006; Waku-Kouomou et al., 2010; Waku-Kouomou et al., 2007;
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14 98 Zandotti et al., 2004]. Although the surveillance of measles is now well established in France,
15
16 99 information regarding SSPE cases is very rare. From 1980 to 1996, around 10 to 30 cases of
17
18 100 SSPE were reported each year by the Renaroug network [Ministère-de-la-santé-DGS,
19
20 101 2008].With the introduction of the vaccination campaign in 1983, the number of measles cases
21
22 102 was reduced drastically and SSPE cases also dropped from 25 in 1980 to 3 cases in 1996.
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24 103 Recently, molecular biology techniques were used to help in the diagnosis of an SSPE case
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26 104 [Souraud et al., 2009]. However, up until the present, there has been no molecular data regarding
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28 105 measles strains causing SSPE in France.
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36 107 The purpose of this study was to describe the detection and molecular characterization of
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38 108 measles viruses isolated in two SSPE cases diagnosed in 1977 (Laine strain) and in 2007 (Hoedts
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40 109 strain) and also to document molecular epidemiological data of measles virus strains in France.
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43 110 In the present study the sequences of the whole coding region of the two SSPE measles virus
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45 111 strains isolated from brain specimens were sequenced and analyzed.
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48 112 Phylogenetic analysis showed that Hoedst strain belonged to genotype C2 while Laine strain
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50 113 could not be related to any known measles virus genotype. The analysis of the whole genome of
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52 114 both SSPE strains revealed biased hypermutations in M, F and H gene. This study describes for
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3 115 the first time molecular characterization of the entire coding region of measles virus isolated
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6 116 from SSPE cases in France.
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3 138 **Material and method**
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6 139 *Patient, specimen, cells and viruses*
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8 140 Brain biopsy specimens obtained from 2 patients were investigated. In both cases, diagnosis was
9
10 141 confirmed clinically, by magnetic resonance imaging (MRI) of the brain or by the presence of
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12 142 measles antibody in cerebrospinal fluid (CSF). Clinical and virological studies information of
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14 143 these cases were published previously [Souraud et al., 2009; Wild et al., 1979].
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20 145 **Patient 1.** A 38 year-old male patient who died 3 months after developing clinical symptoms.
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22 146 The CSF globulin level was elevated, constituting 45.5 % of the total protein. In the brain biopsy,
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24 147 measles antibodies were found. A measles virus strain (Laine strain), was isolated by co-culture
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26 148 of the brain biopsy with vero cells [Wild et al., 1979]. This measles strain was stocked in liquid
27
28 149 nitrogen since his isolation in 1979 and thaw only recently for sequence analysis.
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32 150 **Patient 2.** In a 25 year-old male patient who died after 2 months course of SSPE, the MRI of the
33
34 151 brain showed hyperintensity in the grey matter and the subcortical white matter [Souraud et al.,
35
36 152 2009]. Measles antibody in the CSF was excessively high at 14,000 UI/L. Measles virus
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38 153 sequences were obtained from the brain biopsy by PCR (Hoedts strain).
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41 154 It is assumed that the patients were infected during their childhood. However, virus sequences
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43 155 corresponding to these periods are not available, so the Laine strain was compared to the
44
45 156 Edmonston wild type strain while the Hoedts strain which was a measles virus circulating in the
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47 157 1980s was compared with a wild type strain in the corresponding genotype. Measles virus strains
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49 158 analyzed in this study are summarized in table 1.
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161 **RNA extraction and genome amplification**

162 Viral RNA from SSPE cases was extracted either directly from clinical specimens (brain biopsy
163 for Hoedts strain) or from infected vero cells (Laine strain) using the RNA Now kits (Biogentex
164 Inc, Seabrook, South Carolina, USA) in accordance with the manufacturer's protocol.

165 Measles virus RNA was reverse-transcribed at 42°C for 30 min followed by a denaturation step
166 for 5 min at 85°C using iScript cDNA Synthesis Kit (Biorad, Marnes la Coquette, France). The
167 resulting cDNA was used as a template for PCR amplification of N, P, M, F and H genes-
168 specific sequences. The H gene was amplified as described previously [Kouomou et al., 2002].
169 To amplify F and N genes, PCR were performed starting by a denaturation step at 94°C for
170 5min, followed by 35 cycles of denaturing at 94°C for 30 sec, annealing at 56°C for 45 sec and
171 extension at 72°C for 2 min with a final extension at 72°C for 7min. The PCR cycling program
172 for P and M genes differed from that of F gene by only the annealing temperature, which was
173 59°C for P gene and 55°C for M gene.

174 In order to amplify the L gene, measles virus RNA was reverse-trascribed at 42°C for 90 min
175 followed by a denaturation step for 5 min at 85°C using iScript Select cDNA Synthesis Kit
176 (Biorad, Marnes la Coquette, France). The L gene was amplified as seven overlapping fragments.
177 The PCR Program consisted of a denaturation step at 94°C for 5min, followed by 35 cycles of 30
178 sec at 94°C, 45 sec at 57°C, 2 min at 72°C, with a final extension at 72°C for 7min. Primers
179 sequences used in this study are listed in table 2.

181 **Nucleotides Sequences determination and analysis**

182 PCR products were separated by electrophoresis using a 1.2% agarose gel and then purified
183 using the nucleospin Extract II kit (Macherey-Nagel, Düren, Germany) following the

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3 184 manufacturer's instructions. Sequencing was performed using an ABI 3730 (Applied
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5 185 Biosystems, Langen, Germany). The nucleotides sequences of the N, P, M, F, H, and L gene
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8 186 were aligned and analysed phylogenetically using the Molecular Evolutionary Genetics Analyses
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10 187 (MEGA) software version 4 [Tamura et al., 2007] . Phylogenetics trees were constructed by
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12 188 comparison of the C-terminal part of the N gene and the entire H gene of the sequences derived
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14 189 from the SSPE strains, with the references strains defined by the WHO [WHO, 2005] using the
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16 190 neighbour-joining method. The reliability of each tree was estimated using 1,000 bootstraps
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18 191 replicates. The nucleotide sequences obtained in this study were deposited on Genbank under
19
20 192 accession numbers HM562894-96 (F genes), HM562897-98 (H genes), HM562899-
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22 193 HM562901(L genes), HM562902-04(M genes), HM562905-07 (N genes), HM562908-10 (P
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24 194 genes).
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3 207 **Results**
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8 209 **Phylogenetic analysis**
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10 210 Two SSPE cases were diagnosed in France; one in 1977 (Laine strain) and the other in 2007
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12 211 (Hoedts strain). The coding regions of their entire genome were sequenced, compared to other
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14 212 measles virus sequences available on GenBank and used for phylogenetic analysis.
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17 213 The sequence comparison of the C-terminal part of the N gene with other measles virus
18
19 214 sequences available in Genbank showed that the Laine strain is related most closely to the SSPE
20
21 215 measles strain circulating in the United kingdom in 1956 (Mvs/Belfast1.UNK/1956-SSPE
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23 216 (AF504045) [Jin et al., 2002] and Edmonston strain with 97% identity. Phylogenetic analysis
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25 217 based on the C-terminal part of the N gene showed that the Laine strain could not be assigned to
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27 218 any known measles virus genotype (figure 1). This result was confirmed using the sequence of
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29 219 the entire H gene (figure 2). To assign a new genotype, the minimum nucleotide divergence
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31 220 should be 2.5% for C-terminal part of the N gene (450nt) and 2% for the full length H gene open
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33 221 reading frame from the next most closely related strain [WHO, 2001a]. In this study, the
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35 222 nucleotide divergence between the Laine strain and all measles reference strains was calculated
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37 223 using the nucleotide sequence of the C-terminal part of the N gene and the entire H gene. The
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39 224 results showed that for the N gene, the nucleotide divergence varied from 2.7% with the
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41 225 genotype A to 5.8% with genotypes C2, D10, E, H2. Using H gene, the nucleotide divergence
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43 226 varied from 2,3 % with the genotype A reference strain to 5.5% with genotype H1 reference
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45 227 strain. These results therefore suggested that the Laine strain is closer to the genotype A than to
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47 228 any other known measles virus genotype.
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3 229 The most closely related measles virus to the Hoedts virus was the genotype C2 reference strain
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5 230 and nucleotide divergence was 2.2 and 1,1% for N and H gene respectively. Phylogenetic
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8 231 analysis based on the C-terminal part of the N gene showed previously that it belonged to
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10 232 genotype C2 [Souraud et al., 2009]. These results were confirmed in this study with the H gene
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12 233 (Figure 2). The sequence comparison of the C-terminal part of the N gene with other measles
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14 234 virus sequences available in Genbank showed that Hoedts strain is most close to the measles
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16 235 strain isolated in Canada in 1984 (Monteral.CAN/14.84-AF410973) [Tipples et al., 2004] with
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18 236 98% identity and to the genotype C2 reference strain (Maryland.USA/77), isolated in the USA in
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20 237 1977 [Rota et al., 1994].
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24 238 **Sequence variation in the coding regions of the genome of the SSPE strains**

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27 239 The sequences of the complete coding regions of the genome of both SSPE strains (Laine and
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29 240 Hoedts) were sequenced. They were compared to each other, to measles strains available on
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31 241 Genbank and also with a wild type measles strain of the same genotype or a closely related
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33 242 genotype.
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36 243 Sequence comparison of both SSPE strains showed that, the M gene started by a threonine
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38 244 (Figure 3, Table 3) instead of a methionine usually found as the start amino acids of proteins.
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40 245 Premature stop codons were identified in the F gene (Figure 4) whereas the H gene was
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42 246 elongated in both SSPE strains. Although the majority of mutations found were specific to each
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44 247 strain, common mutations could be identified (Table 3). In the F protein, a mutation I446T was
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46 248 identified. Sequence alignment on Genbank showed that this mutation is present in only 4 of the
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48 249 100 sequences analyzed. In the M gene, a mutation V101A was found whereas in the H protein
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50 250 sequence, mutations R7Q and Y12H were observed in this study. Along the L gene, mutations
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52 251 Y723C and D1887N appear to be specific to both SSPE strains.
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3 252 Due to the fact that the Laine strain could not be assigned to any know MV genotype, the most
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5 253 closely related wild type Edmonston strain, was used for sequence analysis of N, P, M, F, H and
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8 254 L genes. The results showed that the amino acid sequence divergence was 3.6, 3.9, 9.5, 1.8, 3.8,
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10 255 and 1.2% for N, P, M, F, H and L of the Laine and Edmonston strains respectively. Furthermore,
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12 256 a T-C mutation was identified in the P/V gene which results in the replacement of the stop codon
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15 257 of the V protein by a glutamine (Q 300). Therefore, the predicted V protein of the Laine strain
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17 258 may have one more amino acid than the Edmonston strain. In the M gene, the start codon
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19 259 (methionine) was altered to threonine due to a T-C change in the gene. A newly generated
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21 260 termination codon was identified at position amino acid 350 (Table3, Figure 3). Hence, the
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23 261 predicted M protein of the Laine strain may have 15 amino acids more than one of the
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25 262 Edmonston strain (335 amino acids). Another important observation in the Laine M protein
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27 263 sequence was that 66% of the mutations were L-P due to T-C mutation in the M gene. In the F
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29 264 protein sequence, a T-G mutation at nucleotide position7095 results in an earlier termination
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31 265 codon at amino acid position 546 (Table 3). This generates a predicted F protein which may be 4
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33 266 amino acids shorter than the F protein of the Edmonston strain (Figure 4). Sequence analysis of
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35 267 the H gene revealed that there were no termination codons. The attempts to amplify the
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37 268 intergenic region between H and L failed. In the N protein sequence, the Laine strain differs from
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39 269 Edmonston strain by 3.6%. The L protein sequence seems to be more stable than other proteins
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41 270 sequences, with only 1.2 % mutations.

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43 271 The entire coding sequence of the Hoedts was also analysed. As the sequences of the complete
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45 272 genome of the reference strain for genotype C2 (Maryland.USA/77) was not available on
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47 273 Genbank, the genome of the strain M185 [Alla et al., 2006] was sequenced and used as the wild
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49 274 type strain for comparison. The results showed that the amino acid sequence divergence was 1.6,
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3 275 2.3, 6.5, 2.6, 1.4, and 0.9% for N, P, M, F, H and L respectively. The sequence analysis showed
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6 276 that as in Laine strain, the start codon of the predicted M protein of the Hoedts strain was altered
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8 277 to a threonine (Figure 3, Table 3). In the sequence of the F protein, a deletion of a G nucleotide
9
10 278 was detected at position 7031 which generate a reading frame shift that resulted in a premature
11
12 279 termination codon at amino acid position 535 (Figure 4). Taken together, these two evens lead to
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14 280 a predicted altered F protein in the Hoedts strain which may be 11 amino acids shorter than the
15
16 281 one of the wild type strain of the same genotype, the M185 strain. In addition, a mutation M94V
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18 282 was found in the F protein sequence. The analysis of the H sequence revealed that the
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20 283 termination code is located at position 622 instead of 618 as in M185 strain (Table 3). Therefore,
21
22 284 the H protein of the Hoedts strain may have 4 amino acids more than M185 strain. In the N
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24 285 protein, there was only 1 mutation (S427N) in Hoedts strain. In contrast, the P protein contained
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26 286 12 amino acids changes. The L protein had only 0.8% mutations.
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6 299 **Discussion**

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8 300 Subacute sclerosing panencephalitis (SSPE) is rare slowly progressive neurological disorder
9
10 301 caused by the persistent infection of human brain by a defective measles virus. Only wild-type
11
12 302 measles virus sequences have been found in SSPE cases [Rima and Duprex, 2005]. It is
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14
15 303 estimated that about 1 out of 100,000 individuals infected by the measles virus will develop
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17 304 SSPE. This study provides for the first time molecular data on SSPE cases from an historical and
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19
20 305 a contemporaneous strains. These data are the basis that will help for a better understanding of
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22 306 measles strain circulation in France.

23
24 307 Phylogenetic analyses from SSPE cases usually indicate the genotype circulating in the
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26
27 308 geographic area where the patient contracted the primary measles infection [Rima et al., 1997].
28
29 309 This characteristic have been used to identify the source of virus strains causing SSPE [Bellini et
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31 310 al., 2005; Forcic et al., 2004; Mahadevan et al., 2008; Miki et al., 2002].

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33
34 311 The Laine strain, suspected to have circulated in France between the 1940s and 1960s was found
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36 312 to be close to an untyped SSPE strain reported previously to be circulating in the UK in 1956 [Jin
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38 313 et al., 2002] . However, the Laine strain, could not be assigned a genotype. These findings
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40 314 suggested that the Laine strain is an untyped historic strain probably circulating in France while
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43 315 Edmonston strain was circulating in the USA (1954).

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46 316 The high nucleotide identity, 97 and 98% between Hoedts strain and measles strain circulating in
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48 317 the USA (Maryland.USA/77) and in Montreal in 1984 (Montreal.CAN/14.84) respectively,
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50 318 confirmed the presumption that the patient would have been infected in the 1980's. Furthermore,
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53 319 the genotype C2 was first detected in Europe in the 1970s where it was considered to be an
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55 320 indigenous genotype and had recently been exported to the USA and Canada [Riddell et al.,
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3 321 2005]. Thus the genotype C2 found is coherent with temporal and geographical distribution of
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5 322 measles virus.
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10 324 It is widely known that biased hypermutations are a hallmark of SSPE measles virus. Mutations

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12 325 in the F protein of SSPE strains have been previously described [Billeter et al., 1994; Cattaneo et

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14 326 al., 1988; Cattaneo et al., 1989]. So far these mutations resulted in three different type of F

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16 327 protein: i) a F protein with an elongated carboxy-terminus tail [Ning et al., 2002] , ii) a F protein

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18 328 with a shortened carboxy-terminus [Billeter et al., 1994; Cattaneo et al., 1989; Ning et al., 2002],

19
20 329 iii) a F protein with unchanged length despite many amino acids changes [Ayata et al., 2007;

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22 330 Ayata et al., 2010; Ning et al., 2002]. In this study, the cytoplasmic tail of the F protein,

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24 331 predicted from sequences analysis of the gene, is altered in both SSPE strains and presented a

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26 332 short tail pattern. However, the extent and mode of alteration was different in each strain. In

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28 333 Laine strain, a premature stop codon was introduced by a point mutation leading to a stop codon

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30 334 at amino acid position 546 while in Hoedts strain, a deletion of a G nucleotide at position 7031

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32 335 was responsible for a reading frame shift which, subsequently results in a premature stop codon.

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34 336 These two different mechanism are similar to those reported previously [Cattaneo et al., 1988;

35
36 337 Cattaneo et al., 1989; Ning et al., 2002]. It was reported recently that the F gene of SSPE viruses

37
38 338 is a major determinant of neurovirulence, [Ayata et al., 2010]. In the same report, it was

39
40 339 suggested that mutation T461I was sufficient to transform a non neuropathogenic wild type

41
42 340 measles virus into lethal virus. In this study, a different mutation I446T was found in the same

43
44 341 region of the F protein of both SSPE strains. This mutation seems to be very rare as it was found

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46 342 in only 4% of sequences available in Genbank. It might therefore be interesting to study the role

47
48 343 of that specific mutation in the propagation of SSPE strains in the brain. A mutation M94V was

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3 344 observed in the F protein sequence of the Hoedts strain. It was reported that the amino acid at
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5 345 position 94, located in the putative heptad repeat C (HRC) domain of the F protein plays an
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8 346 important role in the fusogenicity and glycoprotein interaction of measles virus [Plempner and
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10 347 Compans, 2003]. This mutation has been reported for another SSPE strain (Osaka2), isolated in
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12 348 Japan [Ayata et al., 2007].

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15 349 The results of sequence analysis of the M gene complies with the published data i.e biased
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17 350 hypermutations were found in the two SSPE strains. The initial codon was substituted by a
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19 351 threonine, raising the question of the functionality of the resulting M protein. It might be
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21 352 interesting to analyze the P/M intergenic region to explore whether there is an early start codon
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23 353 for M protein. According to Ayata et al, [Ayata et al., 2002] mutation in the P3' untranslated
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25 354 region can cause increased read-through at the P/M junction and directly affects M gene
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27 355 expression. A late stop codon was found in the Laine strain, suggesting that the M protein is
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29 356 elongated. Similar results were reported previously [Forcic et al., 2004; Jin et al., 2002].
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31 357 However, in contrast to the present study, most of published studies reported truncated M
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33 358 proteins. It has been reported that the V101A mutation, found in the predicted M Protein of both
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35 359 SSPE strains, is sufficient to generate a functionally defective virus assembly [Runkler et al.,
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37 360 2007]. Among mutations found in the M protein sequence of the Laine strain, one was L165P
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39 361 which is known to impair the ability of measles virus to produce cell-free progeny virus [Jiang et
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41 362 al., 2009]. Biased and even point mutations in the M gene are known to render the M protein
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43 363 insoluble, nonfunctional and therefore, impair the ability of the measles virus to produce cell-free
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45 364 progeny virus [Jiang et al., 2009; Sheppard et al., 1986].

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47 365 Compared to the M gene, the H gene of both SSPE strains were less mutated. However, in both
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49 366 SSPE strains, the predicted H protein was longer than those of the wild type strains. In fact, the
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3 367 stop codon could not be found in the Laine strain, suggesting that the predicted H protein in this
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5 368 strain is elongated. It might therefore be interesting to amplify the intergenic region between H
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8 369 and L genes to ascertain whether the termination codon for H protein exists or not. Mutations
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10 370 were found throughout the H protein of both SSPE strains, however, two of them (R7Q) and
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12 371 (Y12H), were shared by both SSPE strains and were reported previously in SSPE strains isolated
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15 372 in the United Kingdom in the 1950s [Jin et al., 2002] The study of their biological role should
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17 373 give more insight onto the pathogenesis of SSPE.

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20 374 In the present investigation, the complete sequences of the N, P, F, M, H and L gene of two
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22 375 SSPE strains were analyzed. The genotypes of measles virus identified in SSPE cases provided
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24 376 information about the circulation of measles strains in France in the 1940-1960s and 1980s.
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27 377 Sequences analyses results showed that the N, P and L genes had no exceptional mutations. In
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29 378 contrast, striking alterations were observed in the sequence of M protein which has an altered
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31 379 start codon, in H protein where elongated C-terminal tail was found and in the F protein which
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34 380 was partially deleted. Detailed virological and immunological studies will be necessary to
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36 381 explore the biological impacts of these mutations for a better comprehension of SSPE
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39 382 pathogenesis.

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44 45 385 **Acknowledgments**

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Table 1: Measles virus strain analyzed in this study

Measles virus strains	Lab name	Description	Genotype	Genebank Accession Number
Edmonston-wt-USA/54	Edmonston	Wild type MV strain isolated in USA in 1954	A	AF266291
Mvi/Lyon.FRA/77 ^a	Laine	SSPE MV strain isolated in France in 1977	unknown	This study
Mvs/Toulon.FRA/08.07 ^a	Hoedts	SSPE MV strain isolated in France in 2007	C2	This study
Mvi/Temara.MOR/24.03 ^b	M185	Wild type MV strain isolated in Morocco in 2003	C2	Alla et al., 2006, and this study

^a Entire viral genome sequenced in this study

^b Entire viral genome sequenced in this study except H gene (alla et al, 2006)

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Table 2: Sequences of primers used for PCR amplification

Gene	primers name	Primers sequences	PCR product size (pb)
N	N1 bis(+)	5' GATCCTATTATCAGGGACAAGAGC3'	1650
	N4 bis(-)	5' GATGTTGTTCTGGTCCTCGGCCTC3'	
P	P5's2(+)	5' GGAAGATCTTCCAGCCAACCAACCATC3'	1014
	Pseq 3(-)	5' GATGTCCTTGGACATCGGAGAAC3'	
	Pseq2(+)	5' TGTGAGCAATGCCGCACTGATAC3'	730
	P3'(-)	5' GAAGATCTTCCGGCAGGTAAGTTGAGC3'	
M	M1(+)	5' CTTAGGAGCAAAGTGATTGCCTC3'	582
	M2(-)	5' GACCGATCTGAATTCCAGCATT3'	
	M3(+)	5' GTTAATCTGATACCGCTCGATACC3'	633
	M4bis(-)	5' CGCTTGGTCCGTGGAGTCTTTTCG3'	
F	MF1(+)	5' CCCAGAATCAAGACTCATCC3'	880
	MF2(-)	5' CGTCGGATAGGCTATACTGAGGAC3'	
	MF3(+)	5' GGCATCTTAGAGAGCAGAGG3'	932
	MF4(-)	5' CGAAGAGGAGACTTGTGGGAAC3'	
H	gh004(+)	5' GTGCAAGATCATCCACAATGTCACC3'	1251
	mh1251(-)	5' CGTATGAAGGAATCCTGTTATC3'	
	gh1029(+)	5' CCAACCGACATGCAATCCTGG3'	914
	mh1922(-)	5' GTATGCCTGATGTCTGGGTGAC3'	
L	L1s(+)	5' GTGAAATAGACATCAGAATTAAG 3'	1092
	L1as(-)	5' GTCAGATGTATGTCATCAGTTATG 3'	
	L2s(+)	5' GCTTTACTGAAATACATGATGTTCTTGAC 3'	1127
	L2as(-)	5' GCCTCTGTGCAAACAAGCTGATGGTC 3'	
	L3s(+)	5' GACCAAGACACTGATCATCCG 3'	1121
	L3as(-)	5' GAGGAGTCTAGTGATGCTCTGGACACATAC 3'	
	L4s2(+)	5' GATTCTCGCCTCACTAATGCC 3'	718
	L4as2(-)	5' GTTTCCTTGTCAATATCATCCAG 3'	
	L4s3(+)	5' GTGTGGATCAGTCAACTACG 3'	911
	L4as3(-)	5' GAATAATCTTGGCTCTATGAGC 3'	
	L5s(+)	5' GCTAAGTCCACAGCACTATCTATG 3'	1094
	L5as(-)	5' CTCTTTATAAGTGATCAACATAGAACC 3'	
L6s(+)	5' CTGTTGAGATATCAACATTAATTAGGAG 3'	1308	
L6as(-)	5' GCAAATAATGCCTAACCACCTAGGGCAG 3'		

(+) and (-) indicated respectively the forward and the reverse primers

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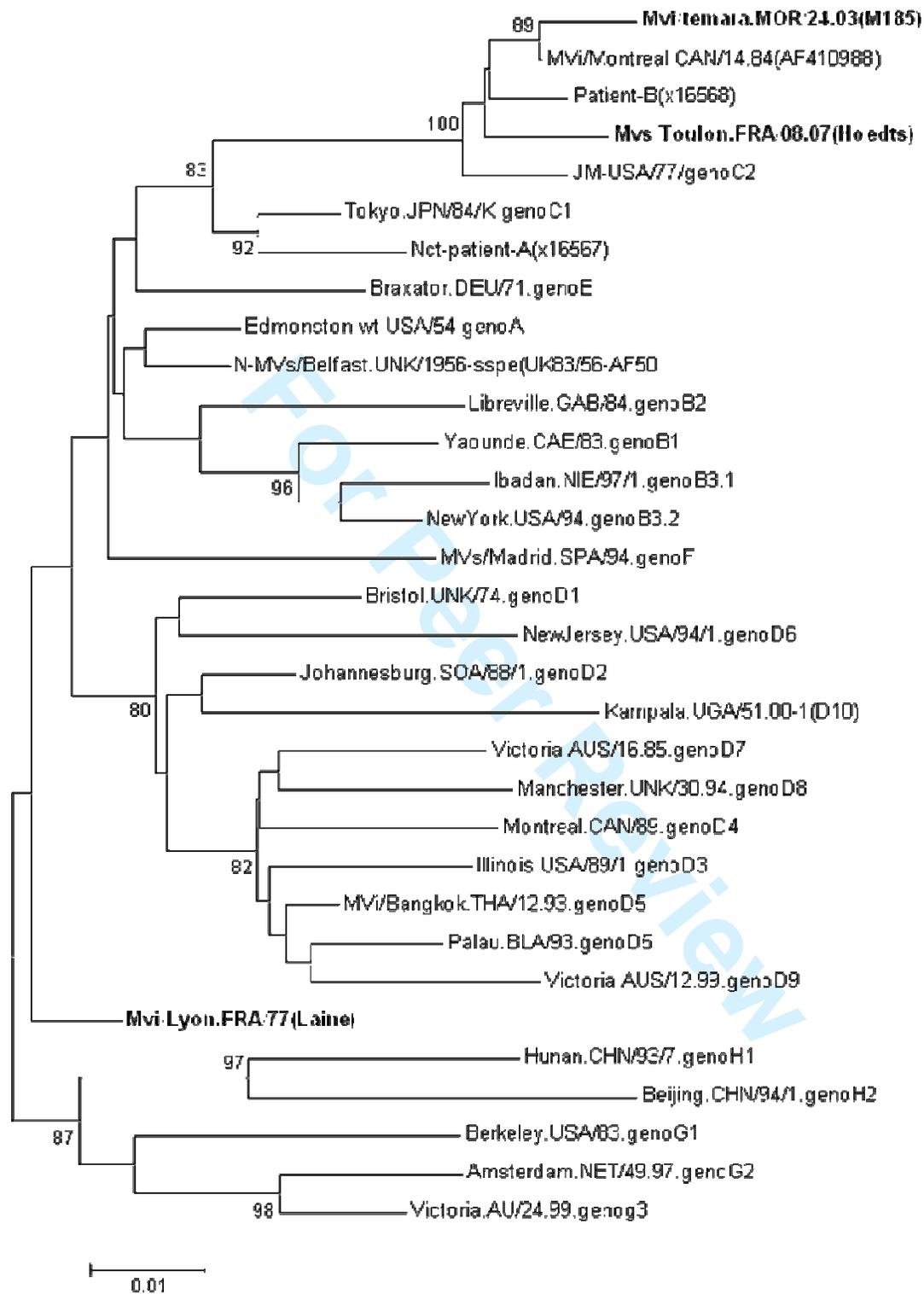
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Table 3 : SSPE stains specific amino acid mutations

Gene	Amino acid position	Edmonston strain (Genotype A)	Laine strain (SSPE)	M185 strain (Genotype C2)	Hoedts strain (SSPE)
P/V	225	G	E	G	E
	300	stop	Q	stop	stop
M	1	M	T	M	T
	5	Y	H	Y	H
	65	L	P	L	P
	97	L	P	L	P
	101	V	A	V	A
	135	F	L	F	L
	165	L	P	L	L
	170	Y	H	Y	H
	180	F	L	F	L
	232	Y	H	Y	H
	248	F	S	F	S
	291	L	P	L	P
	303	V	A	V	A
	335	stop	Q	stop	stop
	350	-	stop	-	-
F	94	M	M	M	V
	449	I	T	I	T
	535	L	P	L	stop
	546	Y	stop	Y	-
H	7	R	Q	R	Q
	12	Y	H	Y	H
	618	stop	Q	stop	W
	622	-	?	-	stop
L	723	Y	C	Y	C
	1887	D	N	D	N

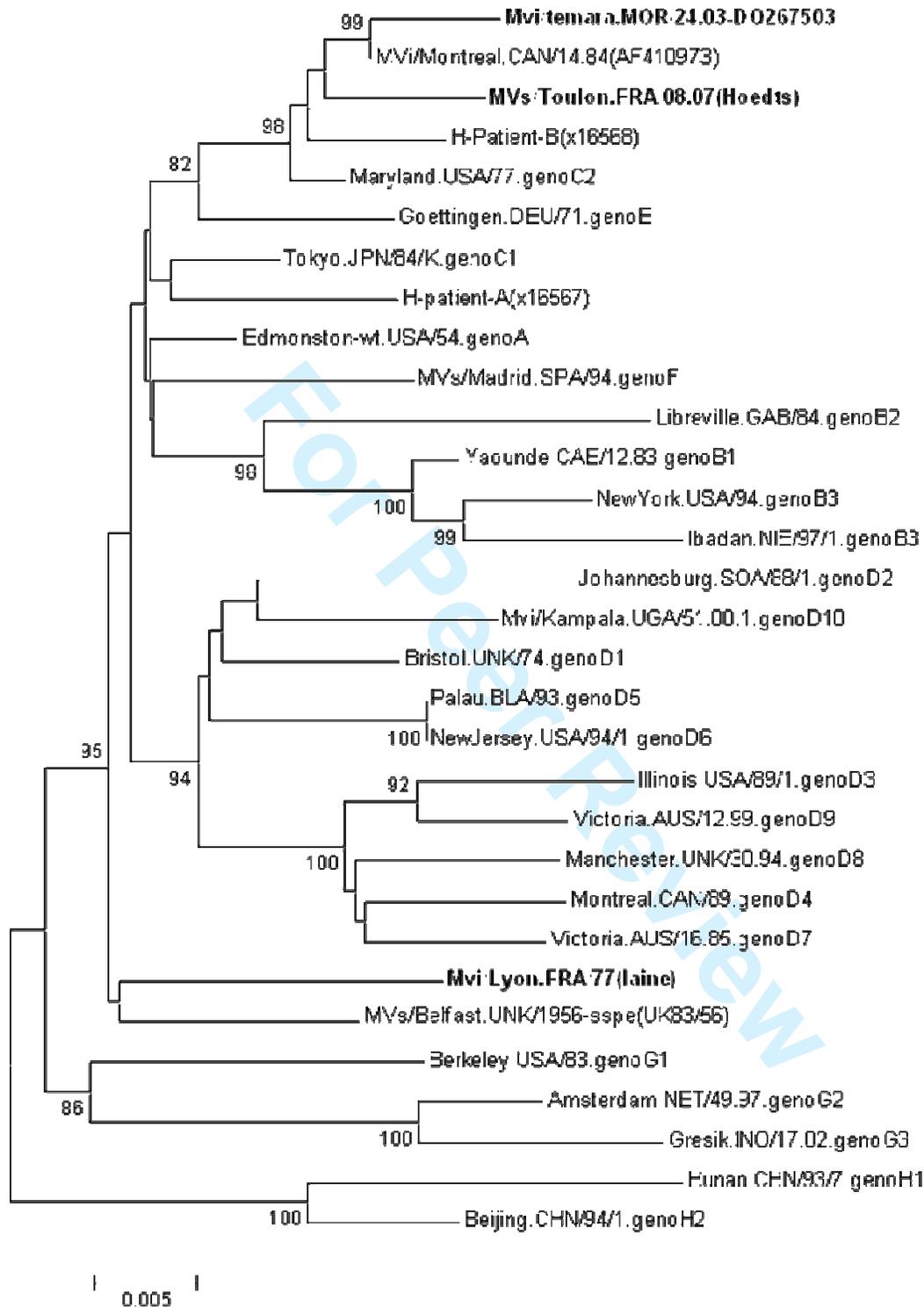
Important mutations specific to only one of the two SSPE strains are indicated in bold. The question mark (?) represented unanalysed amino acid.

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537 **Figure 1:** Phylogenetic tree based on the sequences of the hypervariable region of the N gene, showing the two
 538 SSPE strains (Laine and Hoedts) isolated in France in 1977 and 2007 respectively. Other SSPE strain (Patient B) and
 539 Wild type measles strains (M185) of genotype C2, were also included. Sequences analysed in this study are in bold.
 540 Significant bootstrap values (>80) are indicated.



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542 **Figure 2:** Phylogenetic tree based on the sequences of entire H gene, showing the two SSPE strains (Laine and
 543 Hoedts) isolated in France in 1977 and 2007 respectively. Other SSPE strain (Patient B) and Wild type measles
 544 strains (M185) of genotype C2, were also included. Sequences analysed in this study are in bold. Significant
 545 bootstrap values (>80) are indicated.

