



Title	Progressive multifocal leukoencephalopathy with mild clinical conditions and detection of archetype-like JC virus in cerebrospinal fluid
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3 **Progressive multifocal leukoencephalopathy with mild clinical conditions and detection of**
4 **archetype-like JC virus in cerebrospinal fluid**

5

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35 **Author contributions**

36 Kosuke Iwami, Masaaki Matsushima, Azusa Nagai, Shinichi Shirai, Sho Nakakubo, Ikuko

37 Takahashi-Iwata, Masafumi Yamada, and Ichiro Yabe collected and interpreted the clinical data. Kazuo

38 Nakamichi completed virological analyses. Kosuke Iwami and Kazuo Nakamichi wrote the manuscript and

39 prepared the figures. Kosuke Iwami and Kazuo Nakamichi contributed equally to this work. All authors

40 have critically revised and approved the manuscript.

41

42 **Abstract**

43 Progressive multifocal leukoencephalopathy (PML) is a demyelinating disease of the central nervous
44 system with a poor prognosis and is primarily caused by JC virus (JCV) with a mutation called prototype.
45 We encountered a case of PML with moderate progression and analyzed the mutational patterns of JCV in
46 the cerebrospinal fluid (CSF). A 19-year-old Japanese woman with mild neurological symptoms was
47 diagnosed with combined immunodeficiency following pneumocystis pneumonia. Brain magnetic
48 resonance imaging scan showed multiple brain lesions, and real-time polymerase chain reaction testing
49 detected JCV in the CSF, leading to the diagnosis of PML. The disease course of PML was stable after
50 administration of mefloquine and mirtazapine with immunoglobulin replacement therapy. In the JCV
51 genome cloned from the patient CSF, DNA sequences of the gene encoding the capsid protein (VP1) and
52 the non-coding control region exhibited small mutations. However, they were quite similar to those of the
53 archetype JCV, which persists asymptotically in healthy individuals. These findings provide insight into
54 the mutational characteristics of JCV in PML with mild symptoms and progression.

55

56 **Keywords**

57 Progressive multifocal leukoencephalopathy, JC virus, Non-coding control region, VP1 gene, Combined
58 immunodeficiency

59

60 **Introduction**

61 Progressive multifocal leukoencephalopathy (PML) is a demyelinating disease of the central nervous
62 system caused by JC virus (JCV) in the context of immunodeficiency or immunosuppressive therapy
63 (Cortese et al. 2021; Hadjadj et al. 2019). JCV establishes persistent asymptomatic infections in peripheral
64 sites, such as the kidney and lymph nodes in humans (Cortese et al. 2021). The non-neuropathogenic,
65 persistently infectious form of JCV, which can be detected in the urine of healthy individuals, is called an
66 archetype and has a consistent nucleotide sequence within the viral genome (Yogo et al. 1990). In contrast,
67 JCV isolates from the brain and cerebrospinal fluid (CSF) of PML patients commonly exhibit hypervariable
68 mutations in the non-coding control region (NCCR) of the viral genome (Cortese et al. 2021; Gosert et al.
69 2010; Reid et al. 2011). Prototype JCV also often shows several types of mutations in the gene encoding a
70 surface protein on the viral capsid (VP1) that affect its receptor specificity to host cells (McIlroy et al. 2019;
71 Reid et al. 2011). Here, we report a case of atypical PML with mild progression and symptoms, and
72 analyzed the nucleotide sequences of the VP1 gene and NCCR of JCV in the CSF.

73

74 **Methods**

75 **Patient**

76 A 19-year-old female visited our hospital complaining of difficulty in speaking and using her right limbs
77 for the past month. She had a history of herpes zoster in elementary school and pyelonephritis in high
78 school. There was no family history of neurological disorders or immunodeficiency, except for her
79 grandfather's suspected amyotrophic lateral sclerosis. Neurologically, she had mild dysarthria, mild
80 paralysis of her right upper and lower limbs, and ataxia of her left lower limb. Brain magnetic resonance
81 imaging (MRI) scan showed multiple T2 and fluid-attenuated inversion recovery (FLAIR) hyperintense
82 lesions without contrast enhancement in the cerebral white matter, brain stem, and cerebellum, and the
83 cerebellar lesion was crescent-shaped (Fig. 1a-c). Her CSF was positive for oligoclonal bands. She was
84 fulfilled the 2017 McDonald criteria and diagnosed with multiple sclerosis (MS) (Thompson et al. 2018).
85 However, 2 months after the first visit, the patient developed fever and cough. We prescribed dimethyl
86 fumarate, but she had never taken the medication because of suspicion of infection. One month later, she
87 was admitted to our hospital for a thorough examination of fever and cough. Chest computed tomography
88 scan showed diffuse ground-glass opacities, and markedly high serum beta-D glucan levels (251.8 pg/mL)

89 were detected. We made a clinical diagnosis of pneumocystis pneumonia and started
90 sulfamethoxazole/trimethoprim therapy. She also had low immunoglobulin levels (IgG 137 mg/dL, IgA 30
91 mg/dL, and IgM 97 mg/dL), reduced response in the mitogen-induced lymphocyte proliferation test, and
92 negative anti-HIV antibody. We diagnosed her with combined immunodeficiency and started
93 immunoglobulin replacement therapy. A repeat brain MRI scan showed expansion of the lesions in the left
94 centrum semiovale and left middle cerebellar peduncle compared with those at the initial visit, but these
95 lesions remained without contrast enhancement (Fig. 1d). FLAIR lesions showed ring-shaped
96 hyperintensities in diffusion-weighted imaging (data not shown). Spine MRI showed no intraspinal
97 abnormal signals or abnormal enhancements suggestive of demyelinating diseases such as MS or
98 neuromyelitis optica. CSF cell count and protein levels were within normal ranges. However, the CSF
99 specimen was positive for JCV DNA, as determined by quantitative real-time polymerase chain reaction
100 (PCR) testing (940 copies/mL). Based on the above findings, we re-diagnosed the patient with PML and
101 believed that the first diagnosis of MS was wrong. After obtaining approval for off-label use from the
102 institutional review board of our hospital, we started combination treatment with mefloquine and
103 mirtazapine in addition to immunoglobulin replacement therapy at 6 months after the onset of symptoms.
104 Follow-up MRI performed 1 month after the start of medication (7 months after the onset of disease)
105 showed transient enlargement of the left centrum semiovale lesion and contrast enhancement (Fig. 1e), but
106 these were not seen in the subsequent period (Fig. 1f). The amount of JCV DNA in the CSF decreased to a
107 very low level of < 20% compared to that in the initial test (175 copies/mL) at 9 months after the start of
108 treatment (15 months after onset). During the follow-up period, her activities of daily living were
109 maintained, and there was no neurological deterioration (Fig. 1g). The patient and her family provided
110 written informed consent for the JCV genome analysis.

111

112 **Sequence analysis of the JCV genome**

113 The study protocol was approved by the Ethical Committee for Biomedical Science of the National
114 Institute of Infectious Diseases (approval number: 1247). Total DNA was extracted from CSF specimens
115 using a QIAamp MinElute Virus Spin Kit (Qiagen, Valencia, CA, USA), and the copy number of JCV DNA
116 in each sample was determined using a real-time PCR assay on the LightCycler 96 platform (Roche, Basel,
117 Switzerland) as described previously (Nakamichi et al. 2019). When analyzing the mutation of the JCV

118 genome in the CSF of the patient, it was difficult to amplify the entire nucleotide sequence using PCR
119 because of the small number of viral copies. Therefore, the VP1 gene and NCCR were amplified, cloned
120 into plasmids, and sequenced using the Sanger method. The complete fragment of the JCV VP1 gene in the
121 CSF DNA was amplified using a pair of primers, VP1-F05 (5'- AAG ATC TGC TCC TCA ATG GAT G -3')
122 and VP1-R06 (5'- AGC TAA TGT TGG TAT GGG GAG AC -3'), and KOD One PCR Master Mix (Toyobo,
123 Osaka, Japan) according to the manufacturer's instructions. PCR products treated with 10×A-attachment
124 mix (Toyobo) were ligated to the pANT plasmid vector using the TA-Enhancer Cloning Kit (Nippon Gene,
125 Tokyo, Japan) according to the manufacturer's instructions. Competent *Escherichia coli* cells (ECOS
126 Competent *E. coli* DH5α; Nippon Gene) were transformed with the ligation mixture and plated on an LB
127 agar medium containing ampicillin. After single colonies were picked up, the nucleotide sequences of the
128 VP1 gene were determined in both directions using the Sanger method with universal primers (5'- TAA
129 TAC GAC TCA CTA TAG GG -3' and 5'- GGA AAC AGC TAT GAC CAT GA -3') and additional primers
130 designed for primer walking (5'- AGC AGT GGA GAG GAC TGT CC -3' and 5'- GGA ACC CAA CAT
131 TCA ACA GG -3'). The JCV NCCR in the CSF DNA was amplified using nested PCR with KOD One PCR
132 Master Mix and two sets of primers reported previously (Nakamichi et al. 2013; Sugimoto et al. 1998). The
133 NCCR fragment was ligated to the pANT vector and sequenced from both sides of the insert using universal
134 primers, as in the analysis of the VP1 gene. The sequence data of the VP1 gene and NCCR were analyzed
135 using the CLC Main Workbench Version 21.0.3 software (Qiagen). The nucleotide sequences were
136 deposited in the DNA Data Bank of Japan and were assigned GenBank accession numbers LC627282
137 (VP1) and LC627283 (NCCR).

138

139 **Results**

140 To investigate the virological features of this case, the VP1 gene and NCCR were cloned from JCV in
141 her CSF specimen at the initial testing time (designated here as Ks-286) and sequenced. In the VP1 gene,
142 four clones were aligned, and all had identical sequences. The Basic Local Alignment Search Tool
143 (BLAST) search revealed that the nucleotide sequence of the Ks-286 VP1 gene had a high identity to JCV
144 isolates 733 B (99.91%) and CY (99.81%), both of which were urine-derived archetypes belonging to type
145 7B. We then analyzed amino acid substitutions in VP1, which have been suggested to be associated with
146 changes in prototype JCV in the affinity and specificity of cellular receptors (Gorelik et al. 2011; McIlroy et

147 al. 2019; Sunyaev et al. 2009). In VP1 of Ks-286, while amino acids L55, K60, D66, S267, and S269 were
148 not substituted and were identical to those of the archetype viruses, differences in E69D of Ks-286 were
149 also seen in the archetype 733 B isolate (Fig. 2a). Nevertheless, a mutation at N265 was found in Ks-286,
150 similar to the prototype JCV NIID11-68 isolate (Fig. 2a). Figure 2b illustrates the pattern of the NCCR
151 sequences of Ks-286 and other JCVs. The nucleotide sequences of the 14 NCCR clones were aligned, and
152 they all had the same sequence. Interestingly, Ks-286 presented the NCCR pattern similar to the archetype
153 CY strain rather than the prototype, with only 13-bp and 9-bp deletions in the regions B-C and F,
154 respectively (Fig. 2b). These results suggest that the JCV detected in this case is very close to the archetype,
155 although it has some prototypical features.

156

157 **Discussion**

158 The details of the mechanisms that define the extent and progression of PML are not well understood,
159 and an integrated clinical and virological approach is beneficial. We present a patient with mild PML as a
160 background disease of combined immunodeficiency and the characteristics of JCV detected in the CSF. In a
161 recent retrospective observational study of PML patients with underlying primary immunodeficiency, the
162 median time from diagnosis to death was 8 months, and 5 out of 11 patients died within 6 months of
163 diagnosis (Hadjadj et al. 2019). In our case, the patient was initiated on immunoglobulin replacement
164 therapy and combination therapy of mefloquine and mirtazapine at 4 and 6 months after the onset of PML,
165 respectively. During the follow-up period, no neurological deterioration was observed. The temporary
166 enlargement of the lesion and the contrast-enhancing effect at 7 months after onset may indicate an
167 inflammatory response and clearance of JCV. These were not observed in the later stages of the disease.
168 Therefore, compared with previously reported cases, the disease progression in this case was slow, and the
169 prognosis was favorable.

170 The mechanism underlying the mild conditions observed in this case is not well understood. However,
171 considering the transient contrast-enhancing effect, it was suggested that local immune response to the
172 virus had occurred. Another reason for the moderation of the disease was that JCV appearing in this case
173 might have archetype-like characteristics based on the VP1 and NCCR sequences. We attempted to amplify
174 and sequence the entire JCV genome, but this was difficult because of the low copy number of viral DNA in
175 the CSF samples. Therefore, we cloned the VP1 gene and NCCR separately. The VP1 of JCV that emerged

176 in this case was a perfect match to that of the archetype JCV 733B isolate, except for a substitution at the
177 265th position from asparagine to lysine among all 354 amino acid sequences. Other study groups have
178 demonstrated that in the prototype JCV, amino acid substitutions from asparagine to aspartic acid, threonine,
179 histidine, or serine can occur at position 265 on the surface of VP1 (Gorelik et al. 2011; McIlroy et al. 2019;
180 Reid et al. 2011; Sunyaev et al. 2009). However, to the best of our knowledge, amino acid substitutions in
181 lysine are rare. Thus, it is possible that VP1, which is not only very close to that of the archetype but also
182 has atypical amino acid substitutions, may be related to the pathology in this case.

183 Another major finding in this case was the lack of complex mutations in the NCCR of the JCV genome.
184 The CSF JCV from the patient can be considered an archetype-like virus in that the region D of the NCCR,
185 which is frequently deleted in a prototype, was retained, and there were only a few deletions in other
186 regions. In PML cases, most CSF JCV isolates are prototypes with variable mutations in the NCCR.
187 However, there have been a few previous reports on the detection of archetypes or archetype-like viruses
188 (Ferrante et al. 2003; Iannetta et al. 2013; Pfister et al. 2001; Seppälä et al. 2017). There have been cases, of
189 long-term survival (Ferrante et al. 2003; Pfister et al. 2001) or short-term fatal outcomes (Iannetta et al.
190 2013; Seppälä et al. 2017) in patients, partly depending on the severity of the underlying disease. These
191 lines of evidence suggest that complex mutations in the NCCR are not necessarily required for the
192 development of PML itself and that some cases have a good prognosis when archetype or archetype-like
193 viruses are detected. In the present case, it was assumed that the NCCR rearrangement did not proceed
194 owing to medication or that the virus did not increase its replication because of its archetype-like nature.

195

196 **Conclusion**

197 We found that archetype-like JCV was detected in a PML case with mild symptoms and progression.
198 These observations serve as a basis for understanding the mutational mechanism of JCV and the
199 pathogenesis of PML.

200

201 **Acknowledgments**

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207

208 **Compliance with ethical standards**

209 The CSF was collected for clinical care, and written informed consent was obtained from the patient and
210 her family for the use of the specimen for research purposes. The study was performed in accordance with
211 the ethical standards of the Declaration of Helsinki after approval from the research institution.

212

213 **Conflict of interest**

214 The authors declare that they have no conflict of interest.

215

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276

277 **Figure legends**

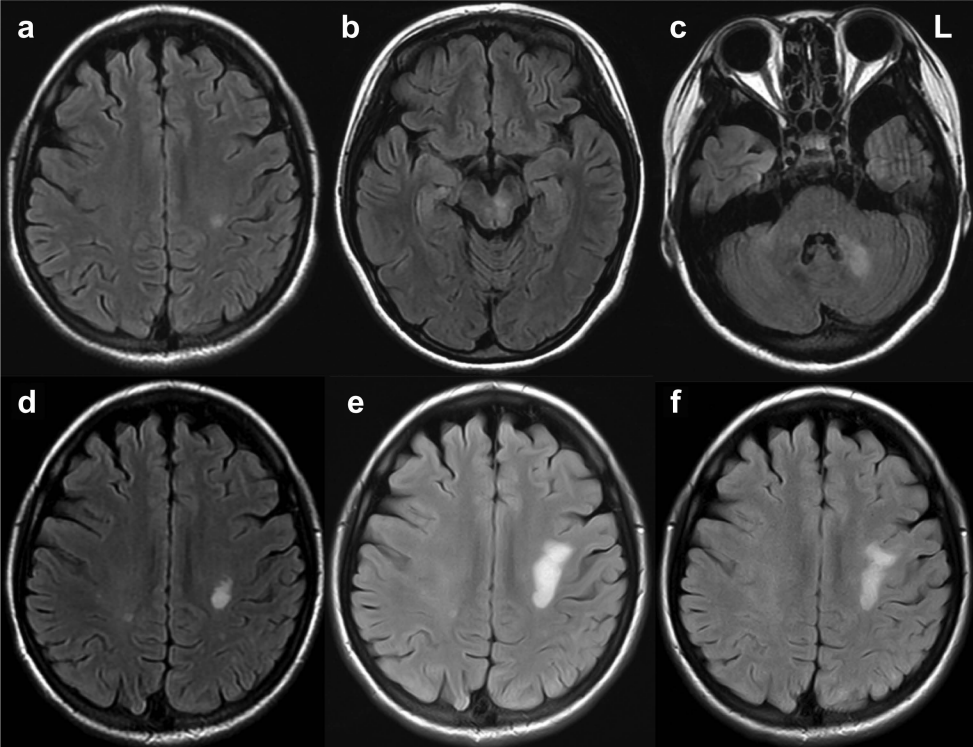
278 **Fig. 1** Brain MRI findings of the patient

279 (a) At the first visit, FLAIR MRI sequences revealed multiple hyperintense lesions in the cerebral white
280 matter, (b) brain stem, and (c) cerebellum. The cerebellar lesion was crescent-shaped. (d) Three months
281 later, the left centrum semiovale lesion had expanded. (e) Six months after the first visit, after 1 month and
282 3 months of concomitant administration with mefloquine and mirtazapine and immunoglobulin
283 replacement therapy, respectively, the lesion temporarily enlarged. (f) Ten months after the first visit, the
284 lesion had stopped expanding. (g) The clinical course of the patient is summarized in a timeline

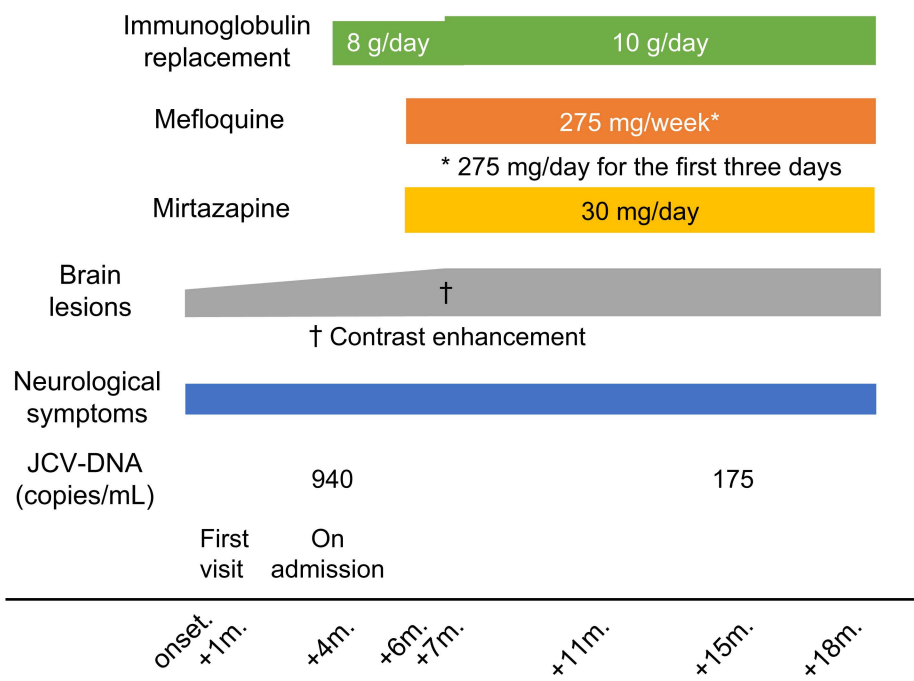
285

286 **Fig. 2** Mutational patterns of JCV in the CSF specimen

287 (a) Alignment of amino acid sequences of VP1 proteins encoded by JCV isolates. The numbers placed
288 down the left side indicate the amino acid positions of VP1. The red background indicates the positions of
289 amino acids located on the surface of VP1 that can be substituted in the prototype JCV. The yellow
290 background illustrates the position where the amino acid difference of VP1 was observed in the JCV isolate
291 of this case (Ks-286) and archetype JCV (733B). (b) Schematic diagram of the nucleotide sequences of
292 NCCR. The NCCR sequences were compared to those of the archetype CY strain, and their patterns were
293 illustrated using *in silico* analysis. The horizontal blue lines indicate DNA sequences identical to the
294 archetype NCCR. The letters A to F are the regions assigned to the archetype NCCR. The numbers above or
295 below the solid line and closed triangles represent the nucleotide positions of the archetype NCCR. The red
296 lines, “Del,” and closed triangles indicate duplication, deletion, and single-base differences, respectively.
297 The GenBank accession numbers of JCV sequences are as follows: AB038249 (CY), AY121912 (733 B),
298 LC627282 (Ks-286, VP1), LC627283 (Ks-286, NCCR), AY536241 (SA296_02), AY536242 (SA28_03),
299 D11364 (Mad11-Br), LC164353 (NIID11-68), AY536240 (SA84_00), AB038254 (Tky-1), AB038255
300 (Tky-2a), and J02226 (Mad-1)



g



a

Archetype (CY) PDEHLRGFSKSI⁵¹SDTFES⁷⁰
 Archetype (733B) PDEHLRGFSKSI⁵⁵SDTF⁶⁹**D**S⁷⁰
 This case (Ks-286) PDEHLRGFSKSI⁶⁰SDTF⁶⁶**D**S⁷⁰
 Prototype (SA296_02) PDEH**F**RGFSKSI⁶⁶SDTFES⁷⁰
 Prototype (SA28_03) PDEHLRGFS**N**SI⁶⁹SDTFES⁷⁰
 Prototype (Mad11-Br) PDEHLRGFSKSI⁶⁶SI⁶⁹**H**TFES⁷⁰

Archetype (CY) DVCGMFTNRS²⁵⁸GS²⁶⁵QQWRGLSR²⁷⁷
 Archetype (733B) DVCGMFTNRS²⁶⁵GS²⁶⁷QQWRGLSR²⁷⁷
 This case (Ks-286) DVCGMFT**K**R²⁶⁵SGS²⁶⁹QQWRGLSR²⁷⁷
 Prototype (NIID11-68) DVCGMFT**D**R²⁶⁵SGS²⁶⁹QQWRGLSR²⁷⁷
 Prototype (SA84_00) DVCGMFTNR**F**GS²⁶⁵QQWRGLSR²⁷⁷
 Prototype (Tky-1) DVCGMFTNRS²⁶⁵G**F**QQWRGLSR²⁷⁷
 Prototype (Tky-2a) DVCGMFTNRS²⁶⁵G**Y**QQWRGLSR²⁷⁷

b