INTRODUCTION

Human herpesvirus 6 (HHV-6) is a neurotropic virus that can establish lifelong latency in a wide range of cell types, including cells of the central nervous system. HHV-6 causes limbic encephalitis due to reactivation in an immunocompromised host and triggers febrile seizures that occasionally evolve into febrile status epilepticus (FSE) in immunocompetent children. In Japan, HHV-6 is a leading pathogen of acute encephalopathy with biphasic seizures and late reduced diffusion, which is among the most common types of acute encephalopathy in children. In adults, HHV-6 has been implicated in the pathogenesis of neurological diseases such as multiple sclerosis and
Alzheimer’s disease. In addition, recent studies have suggested the contribution of HHV-6 to the development of mesial temporal sclerosis (MTS) in patients with temporal lobe epilepsy. Several authors have reported detecting viral HHV-6 DNA in the hippocampus of patients with temporal lobe epilepsy and MTS. A systematic review by Wipfler et al. showed that HHV-6 DNA was detectable in 19.6% of patients with MTS and 10.3% of control subjects, indicating a significantly higher rate of detection of HHV-6 DNA in patients with MTS. HHV-6 remains mostly latent and asymptomatic after the initial infection, but the virus can reactivate and potentially cause various neurological diseases. We encountered a patient with temporal lobe epilepsy associated with MTS following FSE due to HHV-6 infection during infancy. Here, we report the clinical course of the patient and the results of the virological analysis of resected brain tissues.

2 | PATIENT REPORT

The patient was a 14-year-old girl with drug-resistant focal impaired awareness seizures. Her perinatal history was unremarkable until she developed FSE in association with HHV-6 infection at 9 months of age. Two days after the onset of fever, she experienced repetitive seizures with lateral eye deviation and cyanosis followed by generalized convulsions. Impaired consciousness was observed even after an apparent cessation of the seizures. She was transferred to a local hospital. On arrival, she was comatose and no other neurological abnormalities were seen. Blood cell counts were unremarkable and blood chemistry results revealed a mild increase in aspartate aminotransferase to 54 IU/L, a blood glucose level of 181 mg/dL, and mild hyponatremia of 127 mEq/L. The cerebrospinal fluid analysis was unremarkable. Cranial MRI revealed restricted water diffusion in the right temporal area (Figure 1A,B). She was diagnosed with FSE and treated with steroid pulse therapy as well as intravenous phenobarbital and acyclovir. On the fourth day of admission, the fever subsided and a rash appeared on her body, face, and extremities. Later, polymerase chain reaction (PCR) analysis detected $1.6 \times 10^5$ copies/μg HHV-6 DNA in whole blood sampled on admission, but no copies in the cerebrospinal fluid. MRI on day 7 of the illness revealed restricted water diffusion in the right hippocampus (Figure 1C). She was discharged with no apparent neurological sequelae on day 14 of the illness.

At 67 months of age, she had the first unprovoked seizure with loss of consciousness, lateral eye deviation, cyanosis, and left hemiclonus lasting for several minutes. EEG revealed focal spikes in the right temporal area. The MRI scan was first interpreted as normal, whereas MTS was recognized at a later re-evaluation. After the second seizure at 79 months of age, she was treated with carbamazepine (CBZ). Thereafter, no seizures were observed, and the CBZ was discontinued at 131 months of age.

At 145 months of age, she had weekly seizures with impaired awareness along with apparent motor symptoms lasting for 30 sec to several minutes. She also complained of an epigastric sensation before awareness was impaired. No epileptiform discharges were observed on ambulatory routine EEG, whereas MRI revealed the right MTS (Figure 1D,E) and fluorodeoxyglucose-positron emission tomography (18F-FDG-PET) indicated hypometabolism in the right temporal lobe (Figure 1F). She was diagnosed with temporal lobe epilepsy and treated with lacosamide, levetiracetam, and topiramate. The frequency of seizures was not sufficiently reduced by these anti-seizure medications and the patient was referred for surgical treatment.

Nine focal impaired awareness seizures were captured during 1 week of video-EEG monitoring during the preoperative evaluation. All seizures were accompanied by automatism of the mouth and right hand, and dystonic posturing of the left hand. Ictal EEG tests revealed continuous spike waves in the anterior right temporal area preceding each habitual seizure, followed by attenuation in the same area, and eventually, propagated rhythmic activities in bilateral temporal areas. The epileptogenic zone was presumed to be in the mesial temporal structure, and anterior temporal lobectomy was performed at 171 months of age. Severe atrophy was observed in the right hippocampus and lateral temporal cortices. Intraoperative electrocorticography showed frequent spikes in these structures. The anterior temporal structure was completely resected, including the right hippocampus, amygdala, parahippocampal gyrus, and lateral temporal cortices of the 40-mm extension from the temporal tip. MRI revealed right MTS (Figure 1G-I). The postoperative course was uneventful with no neurological deficits such as memory loss. No seizure occurred for 2 months postoperatively. The pathological examination revealed extensive loss of pyramidal...
neurons in Ammon's horn and chromatic agglutination in these neurons.

HHV-6 detection was attempted in resected brain tissues. The one-third of the resected right hippocampus and lateral temporal cortex were stored at −70°C until assay. The two samples (one sample from hippocampus and one from temporal cortex) were homogenized using a Pellet Pestle (Thermo Fisher Scientific Inc., Wilmington, DE, USA). DNA was extracted from each homogenized brain tissue using the QIAamp Blood Kit (QIAGEN, Hilden, Germany). The Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific) was used to measure DNA concentrations. The DNA was stored at −20°C until assay. Real-time PCR was carried out to detect HHV-6 in 10 μL of DNA extracted from each sample. The details of the real-time PCR method have been described previously.\textsuperscript{11} The PCR primers and probe for HHV-6 were selected from the U31 gene (large tegument protein). The PCR reaction was performed using a TaqMan PCR kit (PE Applied Biosystems, Foster City, CA, USA). The detection limit of the assay was 10 copies/tube. HHV-6 DNA was not detected in any brain tissues using the real-time PCR assay. Immunohistochemistry or in situ hybridization against HHV-6 was not performed, because it was unlikely that viral proteins or genome could be detected by these assays.

3 DISCUSSION

The present patient was characterized by typical clinical manifestations of temporal lobe epilepsy due to MTS, including a history of FSE, seizure manifestations,
transient seizure remission, and drug-resistant seizures requiring surgical treatment. MRI and 18F-FDG-PET findings were also compatible with MTS, which was confirmed by pathological examination of a resected brain specimen.

It was remarkable that the initial FSE before MTS had occurred during a virologically proven HHV-6 infection. Thus, our patient provided an opportunity to consider the role of HHV-6 in the development of MTS. In our patient, we found no supportive findings indicating direct viral infection leading to brain damage at the onset of FSE. HHV-6 DNA was not detected in the cerebrospinal fluid during the acute phase of FSE, although the presence of viremia at the onset of FSE was evident based on the detection of HHV-6 DNA in the blood sampled on the same day. Direct evidence to prove contribution of HHV-6 to the development of MTS after FSE was not obtained in our patient, because HHV-6 DNA was not detected in the resected brain tissues. The relationship between MTS and HHV-6 is quite complex. Several studies have suggested the possible contribution of HHV-6 to MTS. We previously reported that HHV-6 DNA was detected in the resected hippocampus tissues of 14 of 52 patients with MTS and 1 of 23 patients without MTS. In addition, the expression levels of monocyte chemotactic protein-1 and glial fibrillary acidic protein were significantly higher in the amygdala of patients with HHV-6 DNA than in those without viral DNA. Theodore et al. detected HHV-6 DNA in fresh brain tissue after surgical treatment of epilepsy in 29 of 54 patients with MTS, 6 of 23 patients with focal cortical dysplasia, and 1 of 3 patients with a history of encephalitis. They also showed that a history of febrile seizure was not associated with the detection of HHV-6 DNA. These data suggest that HHV-6 infection may potentially play a role in the etiology of MTS, but this hypothesis may not be applicable to all patients with MTS. The causal relationship has not been elucidated, as shown by a systematic review. Further studies are necessary to clarify the pathological implications of HHV-6 in MTS.

The MRI findings at the onset of FSE in our patient were remarkable. Reduced water diffusion suggesting cytotoxic edema caused by prolonged seizure activity was evident in the subcortical white matter of the affected temporal lobe immediately after FSE. No hippocampal involvement was seen at the onset of FSE in our patient, but it was observed 1 week later. Yokoi et al. assessed diffusion-weighted imaging (DWI) findings of the hippocampus after FSE and examined their relationship to subsequent epilepsy. DWI revealed unilateral hippocampal hyperintensity in 6 of 22 patients. Five patients with hippocampal hyperintensity exhibited hippocampal atrophy and developed temporal lobe epilepsy 9-13 years later. These results are mostly consistent with the DWI findings in our patient, although the delayed appearance of hippocampal involvement was noteworthy. Several studies have shown the usefulness of neuroimaging findings to predict subsequent epilepsy after FSE. These studies demonstrated that increased hippocampal volume and hyperintensity in T2-weighted images after FSE represent acute injury often evolving into MTS. Recent experimental studies using sophisticated imaging modalities contribute to our understanding of the pathogenesis of MTS after FSE. Choy et al. reported a reduced T2 relaxation time in the amygdala within 2 hours after FSE using 11.7 T MRI in a rat model. T2 changes are presumed to be related to increased oxygen use, which correlates with activation of the intracellular inflammatory cascades previously implicated in epileptogenesis. Sierra et al. compared fractional anisotropy and axial, radial, and mean diffusivities between status epilepticus, traumatic brain injury, and control rats using 9.4 T MRI, and observed increased anisotropy and D || (associated with axonal damage) after status epilepticus. In the future, biomarkers of MTS may be found using imaging techniques.

In summary, HHV-6 DNA was not detected in the resected brain tissues of our patient with MTS after FSE due to HHV-6 infection. The relationship between MTS and HHV-6 is complex, and further studies are necessary to determine the contribution of HHV-6 to the development of MTS.

AUTHORS’ CONTRIBUTIONS
Yoshiki Kawamura: investigation, resources, and writing—original draft (preparation), and editing (equal). Satoshi Maesawa: investigation, resources, and writing—original draft (supporting) and editing (equal). Shingo Numoto: data curation, and writing—review and editing (equal). Ryuta Saito: resources, data curation, and writing—review and editing (equal). Tetsushi Yoshikawa: investigation, and writing—review and editing (equal). Akihisa Okumura: conceptualization, data curation, writing—review and editing (equal), and supervision. We confirm that all coauthors have been substantially involved in the study and/or the preparation of the manuscript; that no undisclosed groups or persons have had a primary role in the study and/or in manuscript preparation; and that all coauthors have seen and approved the submitted version of the paper and accept responsibility for its content.

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CONFLICT OF INTEREST
None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines. We confirm that we have read the journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

DATA AVAILABILITY STATEMENT
Data sharing is not applicable to this article as no data sets were generated or analyzed during the current study.

ETHICS APPROVAL STATEMENT
This study was approved by the institutional review board in Aichi Medical University.

PATIENT CONSENT STATEMENT
Written informed consent was obtained from the patient for publication.

PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES
Not applicable.

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