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SHORT RESEARCH ARTICLE

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Human herpesvirus 6 and epilepsy

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Abstract

We investigated the association between human herpesvirus 6 (HHV-6) and mesial temporal sclerosis (MTS) in 87 patients who had surgery for drug-resistant epilepsy. Fifty-four had MTS, 22 focal cortical dysplasia (FCD), four tumors, three vascular malformations, and three a history of encephalitis. We extracted DNA from fresh brain tissue immediately after surgery and performed viral detection with quantitative real-time polymerase chain reaction (PCR) or digital droplet PCR specific for HHV-6A and HHV-6B. Tissue was studied with standard clinical techniques, including hematoxylin and eosin, glial fibrillary acidic protein, and NeuN stains. Twenty-nine of 54 patients with MTS, six of 23 with focal cortical dysplasia (FCD), and one of three with a history of encephalitis were positive for HHV-6 ($P < .02$). Febrile seizure history was not associated with HHV-6 detection. Patients with MTS had significantly lower seizure onset age than those with other pathologies. Thirteen patients had positron emission tomography with [11C]PBR28, a marker for reactive astrocytes and activated microglia; there was a trend for HHV-6-positive patients to have higher binding in their seizure foci, suggesting inflammation. Our study supports a potential role for HHV-6 in the etiology of MTS.

KEYWORDS

focal epilepsy, HHV-6, mesial temporal sclerosis

1 | INTRODUCTION

The etiology of mesial temporal sclerosis (MTS) in patients with mesial temporal lobe epilepsy (mTLE) is uncertain. Although associated with a wide range of pathologies, including vascular, infectious, neoplastic, and dysplastic, no clear causal relationships have been established.[1] However, data from clinical and imaging studies suggest that MTS is a progressive disease. Increasing focal gliosis, neuronal loss, and hippocampal atrophy are associated with disease duration and seizure burden.[2-3]

A wide range of viruses, including human herpesvirus (HHV) may affect the nervous system and establish latent or persistent infection. HHV-6 is a soluble-stranded DNA virus (~160 Kb); A and B variants have 75%-95% homology.[4] HHV-6-B infection occurs in early childhood (~80% by age two), with most infections only mildly symptomatic, or unrecognized. Ninety percent of US adults have positive serology. Primary CNS invasion occurs, probably via an olfactory route. HHV-6 is associated with limbic encephalitis due to viral reactivation after bone marrow transplantation.

HHV-6 has been associated with complex or prolonged febrile seizures (CFS), and febrile status epilepticus (FSE) has been proposed as a potential antecedent for MTS.[5] In the FEBSTAT study, HHV-6A, HHV-6B, and HHV-7 DNA and RNA were studied with quantitative PCR, and antibody titers were used to distinguish primary from reactivated or prior infection. HHV-6B viremia was present at baseline in 54/169 (32.0%); with 38 primary and 16 reactivated infections.[5] Hippocampal (HF) T2 hyperintensity, maximum in Sommer's sector, occurred acutely after FSE in 22 of 226 children. On follow-up magnetic resonance imaging (MRI), MTS was found in 10 of 14 and HF volume loss in 12.[6,7]

Several studies of tissue resected from patients with drug-resistant epilepsy have suggested that HHV-6 may have a pathophysiologic role.[8-18] However, the results may depend on tissue-processing approaches. We compared detection of HHV-6 in patients with MTS to other epilepsy etiologies using tissue immediately processed after resection.

2 | METHODS

We studied 87 patients who had surgery between 2001 and 2017 (54 with MTS, 22 focal cortical dysplasia (FCD), four tumors, three vascular malformations, and three a history of viral encephalitis (Table 1). Forty-nine had been included in previous studies.[10,11,18] Fourteen had surgery at Children's National Medical Center and 73 at the National Institutes of Health Clinical Center. None of the patients with MTS had a history of cerebral infection, trauma, or a structural lesion aside from MRI evidence of MTS itself.

Presurgical evaluation included ictal video-EEG monitoring, MRI (3T GE SIGNA MRI with fluid-attenuated inversion recovery (FLAIR), T1- and T2-weighted images, and coronal three-dimensional (3D) spoiled gradient recalled (SPGR) acquisition, or 3T Philips MP-RAGE). Thirteen patients had positron emission tomography with [11C]PBR28, a ligand for the translocator protein 18 kilodalton, a marker for reactive astrocytes and activated microglia.[19]

2.1 | Virology

All virological studies were performed at NIH. DNA was extracted from fresh brain tissue immediately after surgery, using previously described procedures.[10,11,18] Primer and probe sequences were NCBI reference genomes NC_001664 for HHV-6A and NC_000898 for HHV-6B and cellular housekeeping gene ribonuclease P protein subunit P30 (RPP30) (Gene ID: 10556).

Forty-four patients had viral detection with quantitative real-time PCR (RT-PCR) and 43 with digital droplet PCR (DD-PCR), both specific for HHV-6A and HHV-6B with sensitivity of 40 viral copies/milliliter.[11, 18]

2.2 | Pathology

Standard pathological studies were performed on resected tissue, including hematoxylin and eosin, antibodies for glial fibrillary acidic protein, and NeuN.

2.3 | Data analysis

We used SPSS (version 19; IBM Inc.) to compare rates of HHV-6 detection across seizure foci, pathology, and clinical features such as a history of complex or prolonged febrile seizures or febrile status epilepticus. We used Student's *t* test for independent sample means as well as analysis of variance. Significance was set at $P < .05$. All virological determinations and pathological studies were performed by investigators blinded to patient clinical, imaging, and electrophysiologic data.

The NIH Combined Neurosciences Institutional Review Board and the Children's National Medical Center Institutional Review Board approved the study.

3 | RESULTS

Patients with MTS were more likely to have HHV-6 detected in surgical resections than those with other pathology. Twenty-nine of 54 patients with MTS, six of 23 with focal cortical dysplasia (FCD), and one with Rasmussen's

TABLE 1 Patient characteristics

Group (n)	Seizure onset age (mean ± SD)	Age at surgery	Duration	Febrile seizures
MTS (54)	9.9 ± 10.2	34.6 ± 12.3	24.3 ± 14.2	16
FCD (23)	13.0 ± 10.1	25.1 ± 8 15	12.1 ± 9.7	1
Encephalitis (3)	12.0 ± 6.1	25.2 ± 14.5	13.2 ± 11.5	1
Tumor (4)	44.0 ± 7.8	48.7 ± 7.8	4.7 ± 5.5	0
Vascular (3)	27.3 ± 8.4	34.3 ± 4.7	7.0 ± 6.0	0

encephalitis were positive for HHV-6 ($P < .02$) (Figure 1). One additional patient (not included in the Figure 1) with a frontal lobe meningioma, anterior temporal findings of single neurons in white matter, and reactive gliosis was positive for HHV-6B in temporal lobe but not in the tumor. Twenty-six patients had HHV-6B, five HHV-6A, and six both viruses detected; there was no difference across pathologic types.

Febrile seizure history was not associated with HHV-6A or B detection, either alone or in combination with MTS status. Sixteen MTS, one dysplasia, and one patient with a history of viral encephalitis had a history of complex or prolonged febrile seizures. Patients with MTS had significantly lower seizure onset age than those with other pathology (10.1 ± 10.2 vs 17.2 ± 13.4 years; $P < .02$). However, among MTS patients, there was no difference in onset age between HHV-6-positive and HHV-6-negative patients.

Three patients (one with FCD) scanned with [11C]PBR28 were positive for HHV-6; all had relatively increased binding in the seizure focus compared to contralateral cortex (mean 7%). Ten patients negative for HHV-6 had variable binding (mean relative decrease 1.5%).

There was no difference in HHV-6 detection between RT-PCR and DD-PCR techniques.

4 | DISCUSSION

This study provides additional support for a role of HHV-6 in the pathophysiology of MTLE-MTS. We found that only a small number of patients with other epilepsy pathology had HHV-6 in resected tissue. A meta-analysis of ten studies through 2016 found that a mean of 19.6% of MTLE patients compared with 10.03% of "controls" including patients with and without epilepsy were positive for HHV-6.[20] The pooled OR of HHV-6-positive cases in MTLE patients was 2.016 [95%-CI: 1.16-3.50]. Our results are based on fresh tissue samples obtained directly at surgery, which may explain why we found a higher proportion of samples positive for HHV-6 DNA than reported by some other investigators.[20] In the largest single study, based on fresh-frozen tissue, there was no difference in HHV-6 detection between patients with MTS and autopsy controls without a history of epilepsy, but MTS patients had significantly higher viral titers.[15] EBV and HHV-6 antigens were detected in 56.7% (17/30) and 50% (15/30) of patients undergoing surgery for Rasmussen's encephalitis compared to none of 16 with CNS trauma.[21]

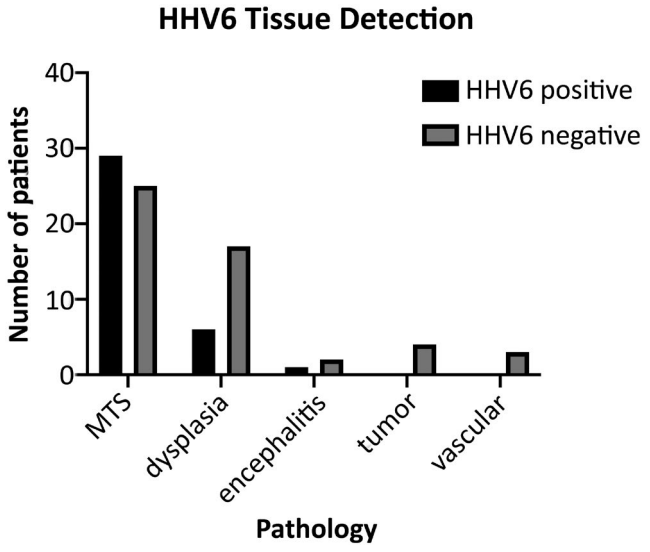

FIGURE 1 HHV-6 detection in resected tissue

Due to the time over which patients had surgery, we were not able to classify pathology according to current International League Against Epilepsy criteria. However, in a previous study including only MTS, we found a nonsignificant trend for HHV-6-negative patients to be more likely to have ILAE type 1.[18]

We did not find an association between HHV-6 detection and a history of CFS/FSE. The majority of our patients were studied as adults. In a meta-analysis, 35.7% of the MTLE patients with detectable HHV-6 DNA had a history of febrile seizure compared to 18.1% in MTLE patients without detectable HHV-6 DNA ($P < .005$).[20]

Twenty-eight studies using a variety of techniques found HHV-6 DNA in 0%-100% (mean 28%) of "normal" brain tissue samples.[22] Using DDpcr, only two of 32 fresh-frozen tissue samples from brain samples of patients who died from nonneurologic illness were positive for HHV-6B, and none of 17 formalin-fixed samples.[23] The data are consistent with establishment of latency but not active replication and pathologic potential, which may require an independent trigger.[22] It is interesting that some of these studies detected HHV-6 at a much higher rate than found in epilepsy surgery studies, particularly for non-MTS patients, or trauma "controls."

HHV-6 could promote epileptogenesis through several potential mechanisms. HHV-6-infected astrocytes show reduced excitatory amino acid transporter activity. Viral load correlated with reduced astrocyte glutamate uptake, potentially leading to glutamatergic excitotoxicity.[11] Inflammation may contribute as well. Activation of innate immunity occurs in MTS and FCD.[4] Increased monocyte chemotactic protein-1, regulating macrophage/monocyte infiltration, and proinflammatory cytokine upregulation have been reported in HHV-6-infected temporal lobe.[4,17] It is intriguing that the HHV-6-positive patients we scanned with [11C] PBR28 had relatively increased binding in their seizure foci, while the negative patients did not. Unfortunately, the groups were not balanced as we could not know who was HHV-6-positive before surgery.

Our results support a potential role of HHV-6 in the etiology of MTLE/MTS. Potential pathways might include activation of preexisting asymptomatic latent infection by an exogenous trigger, or de novo infection that might or might not be associated with CFS/FSE.[5,6,22] However, no direct

causative evidence exists. HHV-6 intranasal inoculation in marmosets accelerated neuroinflammation and development of experimental allergic encephalomyelitis-like symptoms, but not seizures.[24] Nevertheless, considering the severe consequences and frequent drug resistance of MTLE/MTS, it is important to continue to investigate possible etiologies.

CONFLICT OF INTEREST

None of the authors reports any conflicts of interest. 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.

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