

# Search for Human Herpesvirus-6 in Mesial Temporal Lobe Epilepsy Surgical Brain Resections

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## Abstract

Human Herpesvirus-6 (HHV-6) causes roseola infantum and has been associated with multiple sclerosis, seizures, meningitis, and meningoencephalitis. A 2003 study isolated HHV-6 DNA in astrocyte-resembling cells of temporal lobe surgical brain tissue resections in four of eight patients with mesial temporal lobe epilepsy (MTLE). This suggests that the initial infection or reactivation of HHV-6 in astrocytes plays a role in the development of MTLE. We sought to replicate this finding in 18 patients undergoing lobectomy for medically refractory seizures with history of roseola and MTLE. We found no evidence of HHV-6 DNA in temporal lobe specimens of 18 patients with MTLE.

## INTRODUCTION

Human herpesvirus-6 (HHV-6), a T lymphotropic beta-herpesvirus that causes roseola infantum (exanthum subitum), is associated with neurological diseases like multiple sclerosis, seizures, meningitis, and meningoencephalitis.<sup>1</sup> Like other herpes viruses, HHV-6 can cause latent infection of the CNS with the possibility of reactivation.<sup>2,3</sup> HHV-6 DNA was found in the cerebrospinal fluid of immunocompromised patients with limbic encephalitis<sup>4</sup> and in the hippocampus of children with prolonged focal febrile seizures.<sup>5</sup>

## MATERIALS AND METHODS

Donati et al. isolated HHV-6 DNA in astrocyte-resembling cells of temporal lobe surgical brain tissue resections in four of eight patients with mesial temporal lobe epilepsy (MTLE).<sup>6</sup> This suggests that either the initial infection or reactivation of HHV-6 in astrocytes plays a role in the development of MTLE. We sought to replicate this finding using PCR amplification of HHV-6 DNA in pathological brain tissue resected during the surgical treatment of MTLE. Utilizing a larger sample than Donati et al., we studied the relationship between HHV-6A and HHV-6B DNA detected in brain tissue and the reported history of roseola and febrile seizures in patients with MTLE.

Eighteen consecutive patients undergoing temporal lobectomy for medically refractory seizures (10 male, mean

age 38 years, range 19 to 66 years) participated. Mesial temporal sclerosis (11 left, 4 right, 3 without) was detected in most specimens. Table 1 shows demographics, age of seizure onset, and medication.

## Figure 1

Table 1. Hx: History; Sz: Seizure; Infxn: Infection; Mo: Month; MTS: Mesial Temporal Sclerosis; temp: temporal lobe

Patient	Age	Gender	Hx of Febrile Sx	Hx and age of CNS Infxn	Hx of Roseola	Age of Seizure onset (yr)	# Sx/Sz Mo	Medications	Side MTS on MRI	EBD	PCR
1	48	Female	no	no	no	26	4 to 6	phenytoin	Left	L temp	negative
2	21	Female	no	no	no	8	7	carbamazepine, lamotrigine, topiramate, levetiracetam, clobazepam	normal MRI	L temp	negative
3	19	Female	no	yes, 6	no	9	12	carbamazepine	Right	R temp	negative
4	66	Male	no	no	no	13	1	phenytoin, phenobarbital	Right	R temp	negative
5	33	Female	yes	no	no	13	4 to 6	topiramate, lamotrigine	Left	L temp	negative
6	54	Male	no	yes	no	11	5	levetiracetam, phenytoin, topiramate	Left	L temp	negative
7	23	Male	yes	no	no	7	6 to 9	valproic acid, phenytoin, carbamazepine	Left	L temp	negative
8	33	Male	no	no	no	18	7 to 8	levetiracetam, lamotrigine	Left	L temp	negative
9	33	Male	no	yes, 20	no	20	30	carbamazepine	normal MRI	L temp	negative
10	43	Male	no	yes, 7	no	7	16 to 15	levetiracetam, topiramate, diclofenac	Left	L temp	negative
11	21	Male	no	no	no	3	2	Valproic acid, topiramate	Left	L temp	negative
12	36	Female	no	no	no	15	15 to 20	carbamazepine, topiramate	normal MRI	R temp	negative
13	20	Female	no	no	no	18	5 to 10	carbamazepine, topiramate, carbamazepine, valproic acid, levetiracetam	Left	L temp	negative
14	33	Male	no	no	no	0.7	6 to 8	carbamazepine	Left	L temp	negative
15	31	Male	no	no	no	18	2 to 3	topiramate, carbamazepine, levetiracetam, phenytoin, lamotrigine	Left	L temp	negative
16	42	Female	no	no	no	7	16 to 15	carbamazepine, phenytoin, lamotrigine	Right	R > L temp	negative
17	44	Female	yes	no	no	7	10	topiramate	Right	R temp	negative
18	21	Male	yes	no	no	3	6 to 7	Valproic acid, topiramate	Left	L temp	negative

Hippocampal and temporal neocortex resections from the 18 patients were qualitatively analyzed for HHV-6 DNA using nested PCR and gel electrophoresis. Nested PCR assays are generally more sensitive than convention real-time assays, and it had been established that this assay was able to detect to levels less than 10 viral copies/mL. A PCR assay was performed using oligonucleotide primers specific for HHV-6A and HHV-6B. A genomic housekeeping genetic

sequence (beta actin) was amplified and detected to serve as the amplification control for this assay. All PCR reactions were amplified on the Perkin Elmer 9600. Gel electrophoresis was performed on a 6% polyacrylamide gel for 30 minutes at 130 volts.

DNA was extracted from the brain tissue with a Puregene DNA isolation kit, according to manufacturer's instructions (Genra Systems, Minneapolis, MN). Samples had a DNA concentration ranging from 7 to 11ng/uL. A DNA size marker (HAE III cut pBR322, Sigma-Aldrich, St. Louis, MO) was used to determine the presence of the 325 base pair product formed when the HHV-6 virus was present in the sample. The absence of the 325 base pair product indicated the absence of the HHV-6 virus in the sample. Both positive and negative controls for HHV-6 were analyzed in conjunction with the brain samples. Positive controls were DNA samples from previously positive patients as well as plasmids (HHV-6A and HHV-6B strains) manufactured by Advanced Biotechnologies (ABI). A no DNA control was included to ensure the sterility of PCR reagents.

### RESULTS

HHV-6 DNA was not present in any of the samples. Twenty-two percent of the 18 patients had febrile seizures, 22% had seizures beginning with CNS infection, and none had roseola. Mean seizure frequency per month was 10.7 (SD 8.5).

### DISCUSSION

We found no evidence of HHV-6 DNA in temporal lobe specimens of 18 patients with MTLE. Both the hippocampus and temporal neocortex were studied. Our sample included individuals with a history of febrile seizures so that detection of HHV-6 in pathologic sections from these patients would have theoretically strengthened the association between

HHV-6 and MTLE. Our finding is contrary to that reported by the earlier studies of Donati et al.<sup>6</sup> and Uesugi et al.<sup>7</sup> Other infectious or noninfectious causes may have led to the development of MTS in this sample. However, none of the 18 patients had a known history of previous HHV-6 infection. The unreliability of self-report, especially in patients with temporal lobe/memory dysfunction, may affect these results. Future studies should consider the brain tissue of patients with MTLE who also demonstrate HHV-6 antibody in serum or demonstrate a detectable viral load in the peripheral blood.

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