

# Case-Control Study of Primary Human Herpesvirus 6 Infection in Children With Febrile Seizures

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**ABSTRACT.** *Rationale.* Human herpesvirus 6 (HHV-6) has been demonstrated to be the causative agent in roseola infantum. It has been suggested that HHV-6 may have neurotropic properties and be involved in the pathogenesis of febrile seizures in infants. We describe a case-control study to examine the hypothesis that acute HHV-6 infection occurs more commonly in children with febrile seizures than in controls.

*Methods.* Patients presenting with a first or second febrile seizure between 6 months and 2 years of age were entered in the study. Control patients did not have a seizure but had similar inclusion and exclusion criteria. Specimens were obtained for HHV-6 viral serology and polymerase chain reaction in the acute stage and approximately 2 weeks later. A diagnosis of HHV-6 infection was based on HHV-6-specific IgM and IgG serology and HHV-6 polymerase chain reaction of peripheral blood mononuclear cells and saliva.

*Results.* Eighty-six patients (45 with febrile seizures; 41 controls) were enrolled. The HHV-6 infection status could be determined in only 68 patients (35 with febrile seizures; 33 controls). Acute HHV-6 infection was identified in 15 of 35 febrile seizure patients and in 15 of 33 controls. Evidence of past HHV-6 infection was demonstrated in 13 febrile seizure patients and in 8 controls.

*Conclusions.* The incidence of primary HHV-6 infection is similar in patients with febrile seizures and age-matched controls. HHV-6 does not seem to be a major factor in the pathogenesis of first and second febrile seizures. *Pediatrics* 1998;101(2). URL: <http://www.pediatrics.org/cgi/content/full/101/2/e3>; *human herpesvirus 6, febrile seizures, case-control.*

ABBREVIATIONS. HHV-6, human herpesvirus 6; RI, roseola infantum; CSF, cerebrospinal fluid; PCR, polymerase chain reaction; PBMN, peripheral blood mononuclear cells; HSV, herpes simplex virus; CMV, cytomegalovirus; EBV, Epstein-Barr virus; ELISA, enzyme-linked immunosorbent assay; IFA, immunofluorescent assay.

**H**uman herpesvirus 6 (HHV-6), a lymphotropic virus, is the causative agent of roseola infantum (RI).<sup>1,2</sup> HHV-6 infection is more often associated with a nonspecific febrile illness be-

tween 6 months and 2 years of life.<sup>3</sup> Seroprevalence studies in Japan, England, and the United States demonstrate that infection with HHV-6 is common.<sup>4-6</sup> HHV-6-specific IgG can be detected in almost all newborns, but the prevalence declines to less than 10% by 4 to 5 months of age, presumably as maternal antibody wanes.<sup>4</sup> The seroprevalence increases to 65% by 1 year of age<sup>6</sup> and to greater than 90% by 13 to 36 months of age.<sup>4,6,7</sup>

Accumulating clinical and laboratory evidence suggests that HHV-6, like all other herpesviruses, may have neurotropic properties. RI may be complicated by seizures, encephalopathy, aseptic meningitis, meningoencephalitis, and hemiplegia.<sup>8-11</sup> There have also been case reports of HHV-6 associated with aseptic meningoencephalitis,<sup>12-15</sup> encephalitis,<sup>16</sup> and transverse myelitis.<sup>17</sup> Finally, tropism for glial cells,<sup>18</sup> glioblastoma cells,<sup>19</sup> and human fetal astrocytes<sup>20</sup> has been demonstrated using in vitro culture systems.

Febrile seizures are the commonest cause of seizures in early childhood and present before 3 years of age in more than 80% of patients.<sup>21,22</sup> A family history of seizures, usually febrile seizures, is observed in 30% to 50% of cases.<sup>23</sup> A study of 64 same-sex twins with febrile seizures, however, suggested that nongenetic factors might play an important role in the pathophysiology of febrile seizures.<sup>24</sup> Reports of the prevalence of febrile seizures complicating RI have varied considerably from 1% to 50%.<sup>7,25-27</sup> The wide variation in the reported prevalence of febrile seizures complicating RI relates probably to the methodologic differences between studies.

HHV-6 may play a causative role in febrile seizures, based on the high incidence of febrile seizures complicating RI, the neurotropic properties of this virus, and the similarity of age group in which febrile seizures and HHV-6 infection occur. Most previous reports on HHV-6 infection and febrile convulsions have been retrospective or have involved only small patient numbers.<sup>18,19,28-30</sup> We describe a case-control study of HHV-6 infection in children between 6 months and 2 years to examine the hypothesis that acute HHV-6 infection occurs more commonly in children with febrile seizures than in controls.

## METHODS

### Patients

We enrolled children, between 6 months and 2 years of age, who presented with a first or second febrile convulsion between November 1992 and May 1995 at the emergency department of

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British Columbia's Children's Hospital, a tertiary care pediatric hospital. This emergency department also provides a primary care service for the local population who use it instead of a family physician's office. Informed consent was obtained from the parents or guardians of the children.

## Index Cases

The inclusion criteria were: a) first or second febrile seizure; b) a documented temperature of  $>38^{\circ}\text{C}$  in the 24 hours before presentation; c) fever of less than 4 days' duration; and d) agreement by the parent to return in 2 weeks for clinical reassessment and collection of blood and saliva. The exclusion criteria were: a) administration of diphtheria-pertussis-tetanus vaccine or *Haemophilus influenzae* type b vaccine in the 48 hours before the visit, or measles-mumps-rubella vaccine in the 10 days before the visit; b) the presence of a known underlying immunologic disorder; c) a history of blood product administration in the previous 3 months; d) cerebrospinal fluid (CSF) pleocytosis, positive bacterial gram stain, or bacterial culture; e) previous afebrile seizure; and f) evidence of other neurologic disorder or developmental delay. A febrile seizure was classified as complex based on the following criteria: more than 15 minutes' duration, focal features, or more than one seizure in a 24-hour period.<sup>21</sup>

## Control Patients

The inclusion criteria were: a) a documented temperature of  $>38^{\circ}\text{C}$  in the 24 hours before presentation; b) fever of less than 4 days' duration; and c) agreement by the parent to return in 2 weeks for clinical reassessment and collection of blood and saliva. The exclusion criteria were: a) administration of diphtheria-pertussis-tetanus vaccine or *Haemophilus influenzae* type b vaccine in the 48 hours before the visit, or measles-mumps-rubella vaccine in the 10 days before the visit; b) the presence of a known underlying immunologic disorder; c) a history of blood product administration in the previous 3 months; d) CSF pleocytosis, positive bacterial gram stain or bacterial culture; e) previous afebrile seizure; and f) evidence of a neurologic disorder or developmental delay.

## Study Design

Patients were examined at the time of presentation to the emergency department and 10 to 14 days later by a pediatric resident or pediatrician. Acute serum, heparinized blood, saliva, urine, and stool specimens were obtained at this time. Parents were given a diary and asked to record the child's temperature twice a day and to note the development of any additional symptoms, especially that of a rash and the recurrence of seizures. At the follow-up visit, convalescent serum, heparinized blood, and saliva were obtained. All specimens were stored at  $-70^{\circ}\text{C}$  until tested. At the end of the study, the hospital chart was reviewed and the family or family doctor was contacted regarding seizure recurrence.

HHV-6 IgM and IgG were measured in the acute and convalescent sera. HHV-6 DNA was detected by polymerase chain reaction (PCR) in peripheral blood mononuclear (PBMN) cells and saliva, obtained at presentation and at follow-up. Investigations to look for other causes of fever included: a complete blood count; blood culture; urine and stool viral cultures; nasopharyngeal viral culture; and virus-specific IgM and IgG determination of herpes simplex virus (HSV), cytomegalovirus (CMV), and Epstein-Barr virus (EBV). A lumbar puncture was performed at the discretion of the attending physician.

## Laboratory Criteria of HHV-6 Infection

The antibody titer was defined as the reciprocal of the highest serum dilution showing positive fluorescence. Seroconversion was defined as conversion from seronegative to seropositive. An anti-HHV-6 IgG titer  $>1:40$  (range, 1:40 to 1:1280) was deemed positive, and an anti-HHV-6 IgM titer  $>1:40$  (range, 1:40 to 1:640) was considered positive. All HHV-6 IgM-positive sera were titrated up to a 1:640 dilution. The criteria for primary HHV-6 infection were: a) IgM  $\geq 1:40$  in one or both specimens; or b) a four-fold increase in HHV-6 IgG; or c) appearance of HHV-6 IgG for the first time in the second specimen at a titer  $\geq 1:40$ ; or d) seronegativity to HHV-6 in first and second specimens but positive HHV-6 PCR in saliva and/or peripheral blood mononuclear cells. The criteria for past infection were: a) IgM negative and IgG

positive in the first specimen; and b) less than four-fold increase in IgG titer in the second serum sample. The criteria for absence of HHV-6 infection were: both IgM and IgG were negative in the first and second blood specimens, and the PCR was negative in saliva and peripheral blood mononuclear cells.

## Laboratory Methods

### HHV-6 Indirect Immunofluorescent Assay (IFA)

HHV-6 antibody testing was performed by a modification of the method reported by Linde et al.<sup>31</sup> Briefly, CPSR-3 (Sigma Chemicals Co, St Louis, MO) adapted HSB-2 cells (ATCC, Rockville, MD) infected with HHV-6 strain U1102 (ATCC, Rockville, MD) were adhered and fixed in water-free acetone on 8-well glass slides. Cell preparations containing 40% infected cells gave a brilliant fluorescence using anti-HHV-6 IgG control serum at a dilution of 1:1280 and an anti-HHV-6 IgM control serum at a dilution of 1:520. Uninfected HSB-2 cells served as negative controls.

The slides were stored frozen at  $-70^{\circ}\text{C}$  until used. For IgM determination, sera were pretreated with RF absorbent (Behring, Behringwerke AG, Marburg, Germany) to remove interfering IgG. Patient serum samples were initially screened for the presence of anti-HHV-6 IgG and IgM at a 1:40 dilution. If HHV-6 IgM was present, no IgG titration was performed. If both the acute and convalescent serum had HHV-6 IgG but no IgM, the sera were titrated in parallel, using two-fold dilutions to detect an IgG increase. If both the acute and convalescent serum was IgG- and IgM-negative at a 1:40 dilution, but had PCR-positive saliva of mononuclear cells, the sera were retested for HHV-6 IgM and IgG at a 1:20 dilution.

### Enzyme Immunosorbent Assay for Anti-IgG and IgM CMV, HSV, and EBV-Viral Capsid Antigen

Virus-specific IgM and IgG studies used the enzyme-linked immunosorbent assay (ELISA) method with well-characterized, commercially available kits (Behring). Sera for ELISA IgM studies were preabsorbed with RF absorbents (Behring) to remove non-specific IgG. The assays were performed according to the manufacturer's instructions. The test kits all include a noninfected cell control. At the manufacturer's recommended screening test dilution, a positive result is net absorbance (absorbance of antigen test - absorbance of noninfected cell control test)  $>0.2$ , a negative result is net absorbance  $<0.1$ , and an equivocal result is net absorbance = 0.1 to 0.2 after two separate tests.

### PCR for HHV-6

Mononuclear cells from heparinized blood samples and saliva were subjected to PCR. To prepare samples for PCR experiments, 1 mL of heparinized blood was collected and processed fresh or stored at  $4^{\circ}\text{C}$  for less than 24 hours. Mononuclear cells were separated by Ficoll-Hypaque density gradient centrifugation, washed, and stored at  $-70^{\circ}\text{C}$  until tested with PCR. Saliva specimens were obtained and put into a sterile container and transported directly to the laboratory for storage at  $-70^{\circ}\text{C}$  until tested.

A nested PCR procedure was used and the primers were previously described by Kondo et al.<sup>32</sup> The primers were directed against the DNA region coding for a nucleocapsid protein of HHV-6. The outer primers were 5'-GTG TTT CCA TTG TAC TGA AAC CGG T-3' and 5'-TAA ACA TCA ATG CGT TGC ATA CAG T-3'. The inner primers were 5'-CCT TGT GTA GGT GGT CGA ATG CGA C-3' and 5'-ACA GCG CAG CAA CAT GTT TCA GAG C-3'. These primers show no significant homology with HSV type 1 or 2, varicella zoster virus, CMV, EBV, or HHV-7 according to information in GenBank R 68.0 (National Institutes of Health, Bethesda, MD) and EMBL R 27.0 (European Molecular Biology Laboratory, Heidelberg, Germany). The nested PCR assay was able to detect 10 copies of HHV-6 DNA as determined by the titration of purified HHV-6 DNA (strain U1102), provided by Dr. Richard Cone (University Hospital, Zurich, Switzerland). Each sample run included a known positive control consisting of 40  $\mu\text{L}$  of supernatant from HHV-6 (strain U1102) infected HSB-2 cells (30% infection) and 40  $\mu\text{L}$  of supernatant from HHV-6 (strain Z-29) infected HSB-2 cells (10% infection), which had been sonicated 1 minute  $\times$  3, nuclei spun down at 3000 g, and supernatant aliquoted and frozen at  $-70^{\circ}\text{C}$ .

To confirm that amplification conditions were appropriate in

negative specimens, all negative specimens were retested using coamplified sample controls. Purified HHV-6 DNA (155 copies) was added to a second portion of the specimen and retested in parallel with pure specimen using the same PCR protocol. Specimens that gave a negative reaction in the coamplified control were subjected to DNA purification using a commercially available kit GeneClean (BIO 101 Inc, La Jolla, CA) before retesting.

### Virus Isolation

Virus cultures of stool, urine, and nasopharyngeal washings were performed according to standard methods at the Clinical Diagnostic Virology Laboratory at British Columbia's Children's Hospital.

### Statistical Methods

The odds ratio was used to test whether there was a difference in the incidence of primary HHV-6 infection between the febrile seizure group and the control group.

## RESULTS

### Demographics

The demographic data of the patients and controls are shown in Table 1. No focal neurologic abnormality was observed in any patient. The head circumference was normal in all patients. A positive family history of febrile seizures in first-degree relatives was observed more often in the febrile seizure group. Ten of the 30 patients with acute HHV-6 did not have a rash.

### Detection of Serum Antibodies

The results are described in detail in Table 2. On the basis of the HHV-6 serology alone, primary infection was diagnosed in 19 of the index and control patients, past infection in 21 patients, and absence of infection in 25 patients. Of the 19 patients deemed to have primary infection, only 14 had anti-HHV-6 IgM antibodies in one or both samples. The other 5 were initially HHV-6-seronegative but seroconverted in the convalescent sample without developing anti-HHV-6 IgM antibodies at the convalescent visit. No patient had a four-fold increase of anti-HHV-6 IgG antibodies. Of a further 21 patients, deemed to have past infection, none had anti-HHV-6 IgM in either serum and all had anti-HHV-6 IgG antibodies in acute and convalescent sera but no significant titer increase. It was not possible to determine HHV-6 status of the remaining 21 patients by serology alone

because of poor compliance with follow-up (19 patients) and inadequate blood samples (2 patients).

None of the patients had serology suggestive of acute CMV infection; 21 patients had serologic evidence of past CMV infection. All HHV-6 IgM-positive patients were CMV IgM-negative. There was no seroconversion to CMV in the presence of seroconversion to HHV-6. None of our patients had evidence of acute HSV infection; 8 had evidence of past HSV infection. One patient had evidence of acute EBV infection with the presence of IgM on convalescent specimen: this patient's serology was suggestive of past HHV-6 infection. Thirteen patients had evidence of past EBV infection. Therefore, it seems highly unlikely that our acute HHV-6-positive results are falsely positive because of cross-reactivity with CMV, HSV, or EBV infection.

### Nested PCR Analysis of Saliva and Peripheral Blood Mononuclear Cells

The details of these results are outlined in Table 2. We identified a total of 9 saliva and 4 PBMN samples with inhibition by using coamplified sample controls. These were subjected to DNA purification and retesting. Two saliva and 2 PBMN samples turned positive after this treatment. These salivas were both from the same patient, who was in the primary infection group as determined by serology. One of the PBMN samples was a convalescent sample from a patient with past infection, in which the initial sample was already positive. The other was from a patient with past infection, in which the acute PBMN sample was positive. Eleven seronegative patients (8 had two serology specimens, 3 had only acute serology specimens) with a positive PCR result in saliva or PBMN were considered to have primary HHV-6 infection. Based on PCR analysis alone, acute HHV-6 infection was diagnosed in 6 febrile seizure patients and 5 controls. A positive acute PCR was demonstrated (in PBMN, saliva, or both) in 3 patients (1 control and 2 with febrile seizure) who had negative acute serology and no convalescent specimen. A positive PCR (in the acute or convalescent PBMN or saliva specimen or both) was also demonstrated in 4 febrile seizure and 4 control patients, who had neg-

TABLE 1. Demographic Data

	Febrile Seizure (n = 45)	Control (n = 41)
Mean age in months (SD)	15.9 (4.48)	12.27 (4.69)
Sex	21F/24M	17F/24M
Number of patients with otitis media	11	11
Number of patients with upper respiratory tract infection	15	21
Number of patients with lower respiratory tract infection	3	6
Number of patients with urinary tract infection	4	5
Number of patients with bacteremia	3	0
Number of patients with diarrhea during illness	10	10
Average temperature degrees centigrade (SD)	39.69 (0.75)	39.55 (0.14)
Fever duration of 2 days or less at presentation	100%	76%
Number of patients with rash during illness	17	16
Positive family history (febrile/afebrile seizures)	9/9	2/6
Mean age in months (SD):		
No HHV-6 infection (7 seizure; 10 control)	14.70 (5.90)	11.97 (4.92)
Primary HHV-6 infection (15 seizure; 15 control)	15.38 (4.33)	11.43 (5.21)
Past HHV-6 infection (13 seizure; 8 control)	16.5 (4.46)	14.67 (3.85)

**TABLE 2.** Laboratory Basis for HHV-6 Infection Status

	Primary Infection			Past Infection	No Infection	Undetermined
	IgM Positive	IgG Positive <sup>†</sup>	Serology Negative			
Febrile seizures*						
PBMN (+)	1	1	1	2	0	0
Saliva (+)	1	0	2	0	0	3
PBMN and saliva (+)	3	1	3	8	0	0
PCR (-)	1	1	0	3	0	7
Subtotal	6	3	6	13	7	10
Controls						
PBMN (+)	6	1	0	1	0	3
Saliva (+)	2	0	5	0	0	2
PBMN and saliva (+)	0	1	0	7	0	2
PCR (-)	0	0	0	0	0	1
Subtotal	8	2	5	8	10	8

\* (+) = positive; (-) = negative; PBMN = peripheral blood mononuclear cells; PCR = polymerase chain reaction.

<sup>†</sup> Primary infection based on second IgG becoming positive alone. None of the patients had a four-fold increase in IgG.

ative serology on both the acute and convalescent specimens. Thus, based on the serology and PCR studies, 30 patients had acute HHV-6 infection, 21 had evidence of past infection, 17 had no evidence of infection, and the HHV-6 status in the remaining 18 patients was uncertain.

### CSF Analysis

CSF was obtained from 17 patients (15 with febrile seizure and 2 controls). CSF viral cultures were negative in all 17 patients, and HHV-6 PCR was negative in the 16 samples tested. CSF HHV-6 IgG titers were negative in all 8 patients tested. Based on blood and saliva specimens, 6 of these 17 patients had acute HHV-6 infection, 3 had previous HHV-6 infection, 4 had no evidence of HHV-6 infection, and the HHV-6 status was undetermined in 4 patients.

### Patients With Incomplete Specimens

The HHV-6 status could not be determined in 10 patients with febrile seizures and 8 controls. Bacterial and viral culture demonstrated the presence of another organism in 4 of the 10 patients with febrile seizures—bacteremia (n = 1), influenza B (n = 1), and urinary tract infection (n = 2). Bacterial and viral culture demonstrated another infection in 5 of the 8 controls—urinary tract infection (n = 3), adenovirus (n = 1), and respiratory syncytial virus (n = 1). These patients have not been included in the initial statistical analysis as the HHV-6 status was not demonstrated.

### HHV-6 Infection in Febrile Seizure Patients and Controls

Forty-five patients with febrile seizures and 41 controls were enrolled in the study. The HHV-6 status could be determined in only 35 patients with febrile seizures and in 33 controls (Table 3). There

**TABLE 3.** HHV-6 Infection in Febrile Seizure Patients and Controls

	Number	Acute HHV-6	Past HHV-6	No HHV-6 Infection
Febrile seizures	35	15	13	7
Controls	33	15	8	10

was no significant difference in the incidence of primary HHV-6 infection between the controls and the febrile seizure group [odds ratio, 0.90; 95% confidence interval, (0.35, 2.31)]. One of the 4 patients who had a history of a febrile seizure before the study had evidence of past HHV-6 infection at enrollment in the study. Of the 5 patients who had further febrile convulsions after the study, there was laboratory evidence of acute HHV-6 infection in 2 patients and past HHV-6 infection in 2 patients at the time of enrollment in the study.

The HHV-6 status could be determined in all 17 patients with complex febrile seizures but in only 18 of the 28 patients with simple febrile seizures. There was no difference in the incidence of acute HHV-6 infection (7 vs 5) or past HHV-6 infection (7 vs 7), and no HHV-6 infection (4 vs 5) between the patients with simple and complex febrile seizures.

### DISCUSSION

No difference was found in the incidence of acute HHV-6 infection between patients with febrile seizures and controls. The control group matched the febrile seizure group in most parameters examined. A positive family history of febrile seizures was observed more often in the febrile seizure group, consistent with other studies of febrile seizures.<sup>23,24</sup> The degree of fever, type of infection, incidence of rash, and duration of illness was similar between the two groups. The febrile seizure group tended to present earlier in the course of the illness presumably because of concern regarding the occurrence of a seizure. The higher average-age in the febrile seizure group may have contributed to the higher incidence of past HHV-6 infection in that group (37% vs 24%).

Serologic studies are of limited value in the diagnosis of primary HHV-6 infection, because the high anti-HHV-6 IgG seropositivity rate in the general population<sup>4-6</sup> limits the ability of serologic assays to distinguish between primary and reactivated disease. Fox et al<sup>33</sup> described two adults with an acute febrile illness, who had HHV-6 IgG present in baseline samples and yet developed HHV-6 IgM antibodies. He interpreted this as an IgM response in reactivated disease. The exclusion of children >2 years of age in our study, makes reactivation less likely in our

patients. Only 1 of our patients was initially anti-HHV-6 IgG positive and IgM negative. This patient was 7 months old, and it is possible that the anti-HHV-6 IgG antibodies in the initial serum were maternal. The issue of reactivation may be clarified with standardized HHV-6 IgG antibody avidity studies.<sup>34</sup>

False-positive anti-HHV-6 IgM antibody reaction could be the result of cross-reacting IgM antibodies during a primary infection with another member of the herpes group. However, the work of several investigators would suggest that cross-reactivity is rare, and absorption of antibodies to other herpes viruses does not seem to reduce titers to HHV-6 by IFA or ELISA.<sup>35-37</sup> There is no correlation between antibody titers to different herpes viruses and HHV-6 by IFA, ELISA, or immunoblotting,<sup>35,38,39</sup> and the majority of individuals seronegative for CMV and EBV are HHV-6-seropositive.<sup>31</sup> Rising or elevated HHV-6 IgG titers have been described in patients with active CMV or EBV infection.<sup>33,14</sup> This was not a factor in our study group, because nobody had CMV, EBV, or HSV IgM antibodies in the presence of primary HHV-6 infection.

PCR technology offers an alternative method for diagnosis of HHV-6 infection and is particularly suited for fastidious pathogens such as HHV-6. In this study, 2 of 9 specimens from primary infection patients remained PCR-negative despite DNA purification and retesting. This could be attributable to the low sensitivity of the test, pertaining to the primers chosen, or the assay conditions. However, our nested procedure consistently and reliably detected 10 copies of purified HHV-6 DNA, a sensitivity reported by several groups in the field.<sup>40,41</sup> An alternative explanation for low sensitivity is random amplification failures, attributable to the presence of inhibitors in the specimen.<sup>42</sup> This issue was addressed in this study by retesting all negative specimens using coamplified sample controls. In the primary infection group, 6 seronegative febrile seizure patients and 5 seronegative controls were HHV-6 PCR-positive in saliva or PBMN or both. This is likely a function of the sensitivity of the serologic assay. Serology tests may not be sensitive enough to detect very low levels of antibodies or antibodies with low avidity that react poorly in serologic assays.<sup>34</sup> Furthermore, the HHV-6 PCR technique has been reported to be 10 to 10 000 times more sensitive than culture.<sup>32</sup> This supports the possibility that the PCR in some cases is able to detect primary HHV-6 infection before HHV-6 seroconversion.<sup>43,44</sup> It has recently been shown that HHV-7 also causes RI.<sup>45</sup> This finding is not addressed in this article, as it had not been discovered at the time of design of the study and we do not have enough serum to test for this in retrospect. Finally, there is the possibility of false-positive PCR reactions, but we consider this unlikely because of our stringent procedures for preventing DNA contamination and the extensive use of negative controls.

We were unable to determine the HHV-6 status in 18 patients. Nine of the 18 were demonstrated to have another infection. If these 9 patients had only one infection at that time, 1 more febrile seizure

patient would be negative for HHV-6, and 3 more febrile seizure patients and 5 more controls would have past HHV-6 infection. If that assumption were made, there would still be no significant difference in the incidence of acute HHV-6 infection between the febrile seizure and control groups. Indeed, there was no significant difference between the two groups even if all of the febrile seizure patients in whom the HHV-6 status could not be determined were considered to have acute HHV-6 infection, and none of the control patients in whom the HHV-6 status could not be determined were considered to have acute HHV-6 infection.

Based on previous studies the predicted proportion of controls with primary HHV-6 infection was 14%. When the study was designed, we made the assumption that a 50% incidence of primary HHV-6 infection in febrile seizures would be clinically significant. Although we intended to enroll 36 febrile seizure patients and 72 controls, our enrollment of control patients was less than anticipated. We were able to define the HHV-6 status in 35 febrile seizure patients and 33 controls. The failure to find a significant difference in the incidence of HHV-6 infection is not likely attributable to a lack of statistical power; assuming a type 1 error of 5%, we had 90% power to detect a 36% difference in incidence rates. Thus, there is only a 10% chance that we missed a real difference of 36% or more.

Our observation that primary HHV-6 infection is not more common in children with febrile seizures than in controls is consistent with a previous study of the incidence of acute HHV-6 infection in febrile children.<sup>41</sup> In that study, acute HHV-6 infection was observed in 31% of the 67 children with seizures complicating a febrile illness. Among the children under 24 months of age, however, the incidence of first febrile seizures was not significantly different between patients with and without primary HHV-6 infection (13% vs 9%). In addition, a similar incidence of febrile seizures was observed in the 160 children with primary HHV-6 infection and in the 401 matched controls (13% vs 12%).

The incidence of primary HHV-6 infection in acute febrile illness in previous large studies has been reported to be 9%<sup>18</sup> and 14%.<sup>28</sup> In contrast, we observed evidence of acute HHV-6 infection in 44% of the total sample in whom the HHV-6 status could be determined. The higher incidence of acute HHV-6 infection in febrile children in our study compared with other studies probably relates to the study design. In this study, patients were <2 years of age, when primary HHV-6 infection is most likely to occur.<sup>41</sup> Similarly, patients with fever for more than 3 days' duration are less likely to have roseola and were excluded. We also excluded patients with a history of receiving a recent vaccine or blood product and those with evidence of meningitis. Finally, PCR for HHV-6 was used in addition to serology. Thus, we studied a selected population, and our data on the incidence of acute HHV-6 infection are not applicable to a general population of febrile children.

In those for whom the HHV-6 status could be determined, the incidence of primary HHV-6 infec-

tion in patients with febrile seizures in our study (43%) was slightly greater than in other studies. In a study of 1553 children <2 years of age with fever, primary HHV-6 infection was demonstrated in 31% of 67 children with febrile seizures.<sup>41</sup> In another study, 25% of the 42 children had evidence of primary HHV-6 infection, but this study included patients up to 6 years of age and the oldest child with acute HHV-6 infection was 20 months of age.<sup>46</sup> The higher incidence of HHV-6 infection in the present study may relate partly to the use of both serology and PCR for HHV-6 diagnosis. The study design, however, may have resulted in an overestimate of the incidence of acute HHV-6 infection in children with febrile seizures. The inclusion and exclusion criteria were tailored to select a group of patients more likely to have primary HHV-6 infection. The recruitment of all of the patients from a tertiary care pediatric hospital emergency room may also have contributed a bias.

HHV-6 DNA has been detected in the brains of 11 of 13 normal adults, suggesting that it is commonly harbored in the brain in latent form.<sup>47</sup> HHV-6 DNA was also detected by PCR in CSF samples from 7 of 29 children <3 years of age with primary HHV-6 infection, including 2 of 7 samples from children with febrile seizures.<sup>41</sup> HHV-6 DNA was also demonstrated in the CSF of 7 of 8 children who had a lumbar puncture after three or more febrile convulsions and several months after the occurrence of exanthem subitum.<sup>48</sup> It was suggested that HHV-6 may invade the brain during acute HHV-6 infection, establish a latent CNS infection, and cause recurrent febrile convulsions, if reactivated. Our study was not designed to examine the influence of HHV-6 infection on the risk of recurrence of febrile seizures. We did not demonstrate HHV-6 in the CSF of any of the 15 patients with febrile seizures in whom CSF was examined. In addition, only 2 of the 15 febrile seizure patients in our study who were shown to have acute HHV-6 infection had recurrent febrile seizures (median follow-up, 21 months; range, 10 months to 3½ years). Three of the 7 patients recruited at the time of their second febrile seizure had evidence of past HHV-6 infection.

This study has demonstrated no difference in the incidence of primary HHV-6 infection between children <2 years of age with a febrile convulsion and a control group. Although HHV-6 has been postulated to have neurotropic properties, it does not seem to occur more commonly in young children with a first or second febrile seizure than in young children with a febrile illness in the absence of seizure. These data suggest that the occurrence of a first or second febrile seizure is caused by the febrile illness rather than the neurotropic properties of HHV-6.

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