Published in final edited form as:
J Infect Dis. 2010 February 15; 201(4): 505–507. doi:10.1086/650495.

Transplacental Human Herpesvirus 6 (HHV-6) Congenital Infection Caused by Maternal Chromosomally Integrated Virus

Caroline Breese Hall[1,2], **Mary T. Caserta**[1], **Kenneth C. Schnabel**[1], **Lynne M. Shelley**[1], **Jennifer A. Carnahan**[1], **Andrea S. Marino**[1], **Christina Yoo**[1], and **Geraldine K. Lofthus**[2]

[1]Department of Pediatrics, at the University of Rochester School of Medicine and Dentistry, Rochester New York

[2]Department of Medicine, at the University of Rochester School of Medicine and Dentistry, Rochester New York

Abstract

Congenital HHV-6 infection results from germline passage of chromosomally-integrated HHV-6 (CI-HHV-6) and from transplacental passage of maternal HHV-6 infection (TP-HHV-6). We aimed to determine if CI-HHV-6 could replicate and cause TP-HHV-6 infection. HHV-6 DNA, variant type, and viral loads were determined on samples (cord blood, peripheral blood, saliva, urine, hair) from 6 infants with TP-HHV-6 and on their parents' hair. No fathers, but all mothers of TP-HHV-6 infants had CI-HHV-6, and the mother's CI-HHV-6 variant was the same variant causing the TP-HHV-6 congenital infection. This suggests the possibility that CI-HHV-6 replicates, and may cause most, possibly all, congenital HHV-6 infections.

Keywords

Congenital Viral Infection; Human Herpesvirus 6 Infection; Chromosomal Viral Integration

Recently we reported that 1% of newborns have congenital human herpesvirus 6 (HHV-6) infection and that most of these congenital infections (86%) resulted from chromosomally integrated HHV6 (CI-HHV-6) [1,2]. The remainder of the congenital infections were presumed to be due to maternal HHV-6 reactivation or re-infection with subsequent transplacental infection of the fetus, the recognized usual mode of congenital viral infections, as occurs with cytomegalovirus (CMV) [3]. The integration of the viral genome into human chromosomes is a unique mode of congenital infection, and little information exists about the biological characteristics and clinical importance of the integrated virus. Unknown are whether the chromosomally integrated virus replicates and if protective immunity to subsequent HHV-6 infection develops.

Infants with chromosomally integrated virus can produce antibody to HHV-6 [2], which may be in response to the active replication of HHV-6 from a postnatally acquired HHV-6 strain or from the chromosomally integrated virus. The biological implications of this are potentially important in the subsequent outcome and neurodevelopment of infants with CI-HHV-6 as the HHV-6 genome in those with CI-HHV-6 is present in all cell types, including those in the central nervous system [4,5]. We, therefore, postulated that if the chromosomally integrated

Corresponding Author: Caroline Breese Hall, MD University of Rochester School of Medicine and Dentistry 601 Elmwood Ave, Box 689 Rochester, NY 14642 caroline_hall@urmc.rochester.edu.

Potential conflicts of interest: none reported.

virus does replicate, women with CI-HHV-6 could have congenitally infected infants not only from germline passage of the chromosomally integrated virus, but also from transplacental passage of HHV-6.

METHODS

Study Design

We enrolled infants with HHV-6 congenital infection and their parents to study the mechanisms associated with transplacentally acquired congenital infection with HHV-6 [1,2,6]. The families were approached for enrollment only after their private physicians approved of our contacting the family. The study was approved by the University of Rochester Subjects Review Board. Each family provided informed consent.

Children with transplacentally acquired infection were those with HHV-6 DNA detected in cord blood samples but with viral loads which were lower (≤1 genomic equivalent copy (gec) per 10^4 to 10^5 leukocytes, equivalent to <1 gec per μg of cellular DNA [7-9]) than HHV-6 loads associated with chromosomally integrated HHV-6 (CI-HHV-6) [8]. In addition, no child with transplacental HHV-6 infection had HHV-6 DNA in any hair follicle samples. Individuals with CI-HHV-6 were identified by having HHV-6 DNA detected in their hair follicle samples or by the consistent presence of high viral loads of HHV-6 DNA (≥1 gec per leukocyte, or ≥1–2×10^5 HHV-6 gec/μg of cellular DNA), present in all blood samples [2,4,5]. The detection of HHV-6 DNA in hair follicle samples was used to identify CI-HHV-6 among the parents in this study.

Laboratory Analyses

Cord blood, peripheral blood, saliva, and urine samples were obtained from children up to 3 years of age and processed as previously described [2]. The cord blood samples were obtained from free flowing cord blood by procedures specifically designed to avoid maternal blood and other potential sources of contamination. Hair follicle specimens also were obtained from the children and from their parents.

HHV6 DNA Variant Specific PCR Assays—Nested PCR assays with virus-specific typing with oligonucleotide probes specific for HHV-6 variants A and B were conducted as previously reported [2,10]. The assays reliably detected ≤10 genomic copies.

Nested RT-PCR Assays for HHV6—In order to detect replicating HHV-6, our RT-PCR assay amplifies the gp82–105 mRNA of HHV-6 as reported previously [2]. This assay detects <10 mRNA copies.

Quantitative Real-Time PCR Assays—The quantitative PCR assay for the HHV-6 U38 gene was performed as described previously [2]. Mean results from ≥2 wells were reported as genomic equivalent copies of HHV-6 per μg of cellular DNA.

Serologic Assays—HHV-6 antibody levels were determined by an indirect immunofluorescence assay using a clinical HHV-6 isolate which contained HHV-6A and HHV-6B genomes. Positive results were defined as $\log_2$ titers of >3.32 (>1:10 dilution).

RESULTS

We identified six children with transplacentally acquired congenital infection with HHV-6. Three of these children's congenital infection was with variant HHV-6A and 3 were with HHV-6B (table). The HHV-6 viral loads detected in their cord bloods ranged from 2.35 to 4.18

$\log_{10}$ gec per μg cellular DNA, which is characteristic of transplacentally acquired infection and below that observed with infections resulting from CI-HHV-6 (≥5.1 $\log_{10}$ gec per μg cellular DNA) [2]. In the subsequent five peripheral blood samples obtained from the children between 6 and 25 months of age, HHV-6 DNA was detectable in only two samples and at similarly lower levels (table). Of the saliva samples which were collected from the children from 2 to 25 months of age, HHV-6 DNA was identified in 47%, but the HHV-6 DNA was not consistently detected among any child's samples. No HHV-6 DNA was detected in any of the urine samples. Active replication, as detected by the presence of HHV-6 mRNA, was not demonstrated in any of the HHV-6 DNA positive cord blood, peripheral blood, or saliva samples.

Hair follicle samples from the children and from both of their parents were examined for the presence of HHV-6 DNA by PCR. None of the hair follicle samples from the children contained HHV-6 DNA, thus confirming that CI-HHV-6 was not the cause of the congenital infection. None of the hair follicle samples from the fathers contained HHV-6 DNA, but all of the hair follicle samples from the mothers had HHV-6 DNA, indicating that all of the mothers had CI-HHV-6 (table). The rate of CI-HHV-6 (6 of 6) among these mothers of infants with transplacentally acquired congenital infection was significantly greater than the expected rate of CI-HHV-6 among the general population, 1 of 116 ($p<0.0001$, Fisher's exact test) [2].

In further support of the presence of CI-HHV-6 in each mother, the viral loads in the maternal hair samples were all greater than $1–2 \times 10^5$ HHV-6 gec/μg of cellular DNA, and the variant of the HHV-6 DNA detected in the maternal hair follicle in all cases was the same as that detected in the infant's cord blood and in postnatal specimens. One child whose mother had variant A also had HHV-6A in his cord blood and initial saliva sample at 10 weeks of age. However, his subsequent saliva samples after a year of age contained variant B, indicating postnatal acquisition of primary HHV-6B infection.

DISCUSSION

We have previously shown that mothers with chromosomally integrated HHV-6 may give birth to infants with congenital HHV-6 infection acquired by two different mechanisms, germline passage of the chromosomally integrated HHV-6 or by transmission of maternal HHV-6 infection across the placenta [2,11]. However, the findings from the families in this study, though limited, suggest that most, and possibly all, transplacentally acquired infections are associated with the presence of chromosomally integrated virus in the mother.

One explanation for this finding is that the HHV-6 DNA detected in these infants results from the transplacental passage of virus from the maternal chromosomally integrated HHV-6 which is biologically active. Several other explanations should be considered. Transplacentally passed HHV-6 could result from maternal reinfection with a new strain of HHV-6. Alternatively, maternal peripheral blood mononuclear cells containing the integrated virus could be transferred through the placenta from mother to child [12]. Against this, however, is that the HHV-6 DNA was detected in half of the salivas of these children, and since none were breastfed, the source of the detected HHV-6 DNA could not be breast milk. Contamination of the cord blood sample at the time of delivery with maternal blood also is possible. However, not only are our procedures for collection of cord blood samples specifically designed to avoid contamination with maternal blood, but more importantly, we have identified the presence of HHV-7 DNA in the peripheral blood of 67% of pregnant women, yet we have not detected HHV-7 DNA in any of over 2000 cord blood samples examined [1,11].

Transplacental passage of the chromosomally integrated strain is supported by our findings that the HHV-6 DNA variant detected in the mothers was the same as that in their infants, and

that it was variant A in one-half of these cases. HHV-6 variant A is detected significantly more frequently among those with CI-HHV-6 than among the general population of children with postnatally acquired infection [1,2]. Variant A comprises only 1 to 3% of the HHV-6 detected in the blood mononuclear cell and saliva specimens of children and their families [13].

Larger studies of children with transplacentally acquired HHV-6 congenital infection and their families are needed to confirm and expand these observations. Such studies, however, are confounded by the low prevalence of congenital HHV-6 infection resulting from transplacentally acquired virus. Although approximately 1% of individuals have congenital HHV-6 infection [1], only about 14% of them have transplacentally acquired infection or approximately 1 of every 714 newborns [2]. The neurodevelopmental outcome of children with congenital infection from HHV-6 is currently unknown. However, confirmation of these findings would suggest that all HHV-6 congenital infections could be feasibly detected prenatally by determination of HHV-6 DNA in parental hair samples.

Acknowledgments

Financial support:

National Institute of Child Health and Human Development (grant RO1 HD 44430-01), the National Center for Research Resources, National Institutes of Health (General Clinical Research Center grant 5 MO1 RR00044).

References

1. Hall C, Caserta M, Schnabel K, et al. Congenital infections with human herpesviruses 6 and 7. J Pediatr 2004;145:472–477. [PubMed: 15480369]
2. Hall C, Caserta M, Schnabel K, et al. Chromosomal integration of human herpesvirus 6 is the major mode of congenital human herpesvirus 6 infection. Pediatrics 2008;122:513–520. [PubMed: 18762520]
3. Boppana S, Pass R, Britt W. Virus-specific antibody responses in mothers and their newborn infants with asymptomatic congenital cytomegalovirus infections. J Infect Dis 1993;167:72–77. [PubMed: 8380292]
4. Clark D, Nacheva E, Leong H, et al. Transmission of integrated human herpesvirus 6 through stem cell transplantation: Implications for laboratory diagnosis. J Infect Dis 2006;193:912–916. [PubMed: 16518751]
5. Ward K, Leong H, Nacheva E, et al. Human herpesvirus 6 chromosomal integration in immunocompetent patients results in high levels of viral DNA in blood, sera, and hair follicles. J Clin Microbiol 2006;44:1571–1574. [PubMed: 16597897]
6. Caserta M, McDermott M, Dewhurst S, et al. Human herpesvirus 6 (HHV6) DNA persistence and reactivation in healthy children. J Pediatr 2004;145:478–484. [PubMed: 15480370]
7. Qiagen. Nucleic Acids and Proteins: Mammalian Cells and Tissues. 2007.
8. Clark D, Ait-Khaled M, Wheeler A, et al. Quantification of human herpesvirus 6 in immunocompetent persons and post-mortem tissues from AIDS patients by PCR. J Gen Virol 1996;77:2271–2275. [PubMed: 8811027]
9. Jefferies WM, Turner J, Lobo M, Gwaltney J, JM. Low plasma levels of adrenocorticotropic hormone in patients with acute influenza. Clin Infect Dis 1998;26:708–10. [PubMed: 9524849]
10. Hall C, Long C, Schnabel K, et al. Human herpesvirus-6 infection in children: a prospective study of complications and reactivation. N Engl J Med 1994;331:432–438. [PubMed: 8035839]
11. Caserta M, Hall C, Schnabel K, Lofthus G, McDermott M. Human herpesvirus (HHV)-6 and HHV-7 infections in pregnant women. J Infect Dis 2007;196:1296–1303. [PubMed: 17922393]
12. Lo YM, Lau TK, Chan LY, Leung TN, Chang AM. Quantitative analysis of the bidirectional fetomaternal transfer of nucleated cells and plasma DNA. Clin Chem 2000;46:1301–9. [PubMed: 10973858]

13. Hall C, Caserta M, Schnabel K, et al. Persistence of human herpesvirus 6 according to site and variant: possible greater neurotropism of variant A. Clin Infect Dis 1998;26:132–137. [PubMed: 9455521]

Table

Presence and Quantitation of HHV-6 DNA in Specimens from 6 Children with Transplacental Congenital Infection and from their Mothers.

	Children				Mothers[†]
	Cord Blood	**Peripheral Blood**	**Saliva**	**Urine**	**Hair**
No. of Samples	6	5	19	18	6
With HHV-6 DNA:[◆] N(%)	6 (100)	2 (40)[x]	9 (47)[x]	0	6 (100)
Variant: N(% of DNA pos.)					
HHV-6 A	3 (50)	0	0	–	3 (50)
HHV-6 B	3 (50)	2 (100)	8 (89)	–	3 (50)
HHV-6 A and B	0	0	1 (11)	-	0
HHV-6 DNA Load (gec[*]log_{10}/μg DNA)					
mean	3.62	3.14	4.85	0	5.64
(range)	(2.35-4.18)	(2.58-3.38)	(3.83-5.48)	–	(5.30-5.86)
HHV-6 Antibody					
with titer >3.32log_2 N(%)	6 (100)	4 (80)	–	–	–
log_2 titer: mean	7.49	7.82	–	–	–
(range)	(5.32-9.32)	(5.32-9.32)	–	–	–

[†] Hair follicle samples from all children and their fathers were negative for HHV-6 DNA

[◆] HHV-6 mRNA was not detected in any of the blood samples with HHV-6 DNA

[x] The HHV-6 DNA positive bloods were obtained at 6 and 12 months of age and the positive saliva samples from 2 to 25 months of age

[*] genomic equivalent copies per μg cellular DNA