Antiviral therapy of two patients with chromosomally-integrated human herpesvirus-6A presenting with cognitive dysfunction

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ABSTRACT

Background: Human herpesvirus 6 (HHV-6) is a neurotropic virus implicated in central nervous system (CNS) dysfunction, multiple sclerosis, seizures and encephalitis. Inherited or “chromosomally integrated” HHV-6 (CIHHV-6) is a condition characterized by high DNA loads and germ line transmission of HHV-6 genomes, which are integrated into the telomere. Objectives: We previously reported that integrated HHV-6 can be reactivated by trichostatin A in vitro. Therefore, we hypothesized that a broad array of neurological symptoms of CIHHV-6 patients may respond to antiviral drug treatment. Study design: The patients have been treated with antiviral drugs and monitored for viral load, late mRNA, and clinical improvement. Results: Antiviral therapy of two CIHHV patients resulted in successful clinical resolution. However, both patients relapsed on multiple occasions within 4–6 months of cessation of antiviral therapy. Conclusions: Successful antiviral drug treatment suggests that clinical symptoms of these patients were due to symptomatic reactivation of CIHHV-6. Alternatively, some CIHHV-6 patients may have a reduced resistance to community-acquired HHV-6 strains due to tolerance leading to persistent infections.

1. Background

Human herpesvirus 6 (HHV-6) is a neurotropic, lymphotropic, DNA virus with two variants – A and B.1 Like other herpesviruses, HHV-6 persists in latent reservoirs after primary infection. Primary HHV-6 infection is typically mild or asymptomatic in immunocompetent persons, but reactivated HHV-6 has been linked to a wide-range of conditions, including encephalitis,2 seizures,3 myocarditis,4–6 chronic fatigue syndrome (CFS)7–9 and multiple sclerosis.10–13 Unique among herpesviruses, HHV-6 DNA can also occasionally integrate into the human genome and be passed through the germ line.14 Inherited or chromosomally integrated HHV-6 (CIHHV-6) has an overall prevalence of 0.85% in the US15 and 0.8% in the United Kingdom,16 although the prevalence in selected patient populations such as solid organ and stem cell transplant, Hodgkin’s disease and encephalitis patient populations is higher at an average of 2%.17

2. Objectives

In a previous study, we discovered CIHHV-6 in two patients of the current study, one of their parents, and a third sibling, the latter two of whom were asymptomatic.18 We now evaluated two siblings for possible infectious etiologies of their chronic neurological abnormalities.

3. Study design

Two patients with neurological abnormalities have been evaluated for HHV-6 viral load, late mRNA expression, and clinical symptoms before, during and after antiviral drug treatment.
4. Results

4.1. Clinical symptoms and antiviral drug treatment of Patient A

Patient A had a history of asthma, atopic dermatitis, and persistent sinopulmonary and tonsil infections from age 5. At 12 years of age, she experienced three weeks of emotional lability and a sudden onset of severe stuttering which resolved after one week with minor residual speech difficulties. This episode was diagnosed as a seizure. Her EEG during the acute phase was abnormal with diffuse slowing and focal spikes, but an MRI was normal. At age 14, she had an evaluation by an immunologist for the continued sinopulmonary infections and was found to have mild hypogammaglobulinemia of 569 mg/dL (normal control: 694–1618 mg/mL), and impaired T cell response to mitogens (phytohemagglutinin, concanavalin A, pokeweed mitogen) and antigens (candida albicans, tetanus toxoid, mumps), suggesting combined antibody and cell-mediated immune deficiency. She was started on intravenous immunoglobulin (IVIG) for a six-month trial, with a significant improvement in her symptoms. She performed well in school and athletics for the next two years.

However, at age 16, she developed sudden and significant cognitive impairment, hypersomnia, diurnal panic attacks, dysthymia, muscle weakness, and attention deficit disorder-like dysfunction, all requiring a reduction in her school workload to part-time. Extensive testing for these symptoms did not reveal a cause, although a sleep disorder specialist found her plasma interleukin-6 levels were abnormally elevated on two consecutive tests 1 month apart (9.0 and 12.5 pg/mL, normal <5; Specialty Laboratories, Valencia, CA).

She continued to complain of cognitive difficulties and at age 18 she was found to be positive for HHV-6A DNA in her serum (Medical Diagnostic Laboratories, Hamilton, NJ), with 768 copies/mL of HHV-6 U100 RNA in whole blood (qRT-PCR, Virocor; Lee's Summit, MO). A repeat EEG remained abnormal with diffuse slow waves and focal spikes as with the previous EEG at age 12. A repeat MRI was unremarkable.

In the absence of any treatment, by 19 years, her cognitive function had deteriorated further, and she was unable to comprehend complex written or verbal instructions. A trial of foscarnet therapy was planned for presumed HHV-6 CNS infection with 486 copies/mL in CSF (Commonwealth Biotechnologies) and a positive HHV-6 mRNA in whole blood of 871 copies/mL (Virocor). In an attempt to find an additional objective means of evaluating the effectiveness of the foscarnet, a baseline quantitative EEG (QEEG) was arranged. QEEG has been demonstrated to be an objective and quantitative means of measuring cognitive dysfunction and neurological disorders.19–21 The QEEG was consistent with encephalopathy, measuring delta wave velocities that were over three standard deviations higher than those from a database of sex and age matched healthy controls (QEEG, Fig. 1). Excess beta activity in the frontal (F3 F4) regions, significant abnormalities in coherences, and significant power asymmetries within both hemispheres in delta, alpha and beta bands were seen. She underwent 6 weeks of intravenous foscarnet treatment (90 mg/kg, every 12 h) for presumed HHV-6 CNS infection.

Foscarnet treatment was associated with a rapid resolution of hypersomnia, malaise, and cognitive difficulties. A repeat qualitative test of CSF for HHV-6 DNA at three weeks was positive (Wisconsin Viral Research Group, Milwaukee, WI). Repeat QEEG on the last day of treatment showed a drop in delta wave frequency of approximately two standard deviations (from 3.3 to 1.65; Z-score measure at F4) as well as normalization of theta wave.
velocities (Fig. 1 and Supplemental Fig. 1). She returned to school full-time and discontinued all antidepressant and anxiolytic medications. Unfortunately, six months later, her cognitive dysfunction returned. She withdrew from coursework and remained disabled for three years.

At age 23, she began a regimen of oral valganciclovir (900 mg twice daily for three weeks, followed by 900 mg daily), which resulted in a second resolution of symptoms. Pre-treatment whole blood PCR demonstrated $4.5 \times 10^6$ copies/mL (Fig. 2A). Suspected CIIHHV-6 status was confirmed by hair follicle HHV-6 PCR (Red-labs, Reno, NV). The therapy resulted in a gradual resolution of symptoms, but symptoms recurred when the valganciclovir dosage dropped to 450 mg or below. A dose of 1350–1800 mg daily for a sustained period of 18 months resulted in full symptom resolution. Her HHV-6 viral DNA load was consistently less than $2.0 \times 10^6$ copies/mL during this period. Patient A resumed rigorous university coursework and completed her Bachelor’s degree. At age 29, she is working full time as a software engineer and is no longer taking antivirals.

4.2. Clinical symptoms and antiviral drug treatment of Patient B

At four years of age, Patient B – the youngest and previously healthy brother of Patient A – developed a severe clinical depression with neurologic signs (e.g., slurred speech, monotonic voice, ptosis) and intermittent severe anxiety. The acute phase resolved after two weeks, but he continued to suffer from persistent lymphadenopathy, memory impairment, dyspnea, and marked clumsiness.

From age 5–6, he suffered from persistent fungal infections on his skin and abdominal pain from a small bowel bacterial overgrowth with lymphoid nodular hyperplasia in the terminal ileum. At age 6, this prompted immunological testing, and he was found to have insignificant response to tetanus toxoid at 236 counts per minute (CPM) resulting in a stimulation index (SI) of 2.1 (normal >5) and to candida at 142 CPM (1.3 SI, normal >3). He had had poor specific antibody titers to diphtheria and tetanus vaccination (<10 IU/mL and .12 IU/mL). Therefore, together with poor T cells response to tetanus toxoid and candida albicans, like his
sister he also appeared to have combined specific antibody and cell-mediated immune deficiency. He was treated with IVIG for 6 months with partial success. Later, at age 10, impaired specific antibody response was documented against the majority of 14 serotypes of pneumococci following Penumovax-23 immunization.

By age seven, he had developed mild gait and upper extremity ataxia as well as peripheral neuropathy (weakness, decreased vibratory sense) in his hands. He tested positive for HHV-6 U100 mRNA with 772 copies/mL in whole blood (Viracor). His QEEG exam was abnormal, characterized by significant excess power in the theta band in the posterior regions and significant increases in the theta mean frequency, considered a sign of cognitive dysfunction or brain injury.22,23 At age eight, he underwent a lumbar puncture as part of a neurological exam to further explore the etiology of his ataxia and cognitive problems, HHV-6A DNA was found in the CSF (200 copies/mL, Viracor); his MRI was normal.

At age 11, following an asthma attack that required steroid treatment and hospitalization for respiratory distress, his neurological symptoms worsened, with extreme agitation and slurred speech. He had a strong positive on a semi-quantitative serum PCR for HHV-6 (Children’s Hospital Los Angeles) and 500 copies/mL HHV-6 was detected in CSF (Viracor). Seven months of oral valganciclovir (900 mg twice daily for 3 weeks, followed by 900 mg daily) resulted in dramatic clinical resolution of the ataxia, slurred speech, and cognitive impairment.

Four months after discontinuing valganciclovir, however, Patient B relapsed with increasing dyspnea, mild hypoxia (SpO2 90% on room air), cognitive dysfunction, and emotional lability. At this time, HHV-6 was 1.9 × 10⁹ copies/mL by whole blood PCR, which increased to 4.9 × 10⁹ copies/mL six weeks later (Fig. 2). Two months later, at age 12, he developed severe anxiety, and whole blood HHV-6 PCR revealed 1.2 × 10⁷ copies/mL. He restarted valganciclovir (900 mg twice daily for 3 weeks, followed by 900 mg daily), and HHV-6 DNA levels dropped by >2 log₁₀ to 1.03 × 10⁵ DNA copies/mL (Viracor) after three weeks and remained under 1.0 × 10⁶ copies/mL for 4 months. He achieved complete clinical resolution after 3.5 months of treatment.

Four months after the end of his second course of valganciclovir, he again became symptomatic. He performed at the third percentile on the short-term memory index of the Rey Complex Figure Test and Recognition Trial, and he required remedial classes to stay at grade level. His viral load had increased to 3.5 × 10⁶ copies/mL, so a third course of valganciclovir was initiated at age 13. After three months of therapy, Patient B’s viral load decreased to 2.7 × 10⁶ copies/mL and was accompanied with sustained improvement of his symptoms. At age 17, he is in the top 2% of his class, a varsity athlete, and has required no antiviral or asthma medication for four years. His viral load has remained at less than 2.0 × 10⁴ copies/mL (Viracor).

It was retrospectively determined that both siblings had HHV-6 telomeric integration into chromosome 18q23 confirmed by fluorescent in situ hybridization (FISH) in peripheral blood mononuclear cells (as described elsewhere).24

5. Discussion

In this report, we described two CIHHV-6 patients with debilitating CNS dysfunction that responded to therapy against replicating HHV-6A. In both patients, during symptomatic periods, whole blood analyses yielded high levels of HHV-6A DNA persistently greater than 5.5 log₁₀ copies/mL. CSF analysis revealed low copy number (200–600 copies/mL) with no pleocytosis. HHV-6 late mRNA was detected in the serum at approximately 1000 copies/mL (Viracor), suggesting reactivation of the virus. Simultaneous clinical and virologic improvement correlated with administration of the DNA polymerase inhibitors valganciclovir and foscarnet, which have in vitro and in vivo activity against replicating HHV6, unlike acyclovir.24 The response to these medications suggests that suppression of lytic viral replication was responsible for symptom resolution, but the specific cell type that underwent lytic activation is unknown and requires further study.

The patient from whom the CIHHV-6 was acquired is healthy and taking no medications at age 64. Likewise, the two patients also have a healthy CIHHV-6 brother. Future studies are warranted to reveal the underlying causes; it is possible that silencing of the affected chromosomal allele is preventing manifestation of disease in apparently healthy CIHHV-6 individuals.

Although treatment with antiviral therapy resulted in >0.5 log₁₀ reduction in whole blood viral loads for the two siblings in this report, we cannot draw conclusions about a relationship between absolute viral load and clinical status. It is impossible to use current clinical laboratory qPCR data to confirm reactivation of CIHHV-6 due to the high background levels of integrated HHV-6 DNA in these patients. After antiviral treatment one patient had dramatic improvement in cognitive functioning coinciding with normalization of quantitative EEG. This was a retrospective evaluation with significant limitations as there were no objective measures (other than quantitative EEG) for the efficacy of the antiviral therapy. Both patients showed significant improvement in academic functioning that correlated with antiviral therapy.

In future studies, RT-PCR mRNA testing and normalization of viral copies to total cellular DNA mass or to single cellular genes, e.g., albumin,25 beta-globulin16 may provide better characterization. To consider initiating treatment of CIHHV-6 patients with antiviral drugs, the physician must use clinical judgment since meaningful virological supporting evidence is unavailable currently. Significant decline in cognitive and executive functioning26 and the presence of excessive slow wave activity27 – as was seen in the case of these two patients – may suggest virus reactivation in these patients.

We argue that the presence of late HHV-6 mRNA would indicate active replication. When commercial HHV-6 mRNA assays become available we recommend testing peripheral blood for mRNA expression before, during and after antiviral therapy. The blood should be directly drawn in tubes that conserve the integrity of RNA and promptly sent to the laboratory.

We recommend EEG or QEEG as the most objective measure used to determine low level HHV-6 reactivation, as an increase in delta and theta slow wave activity is common in patients with HHV-6 encephalitis.27 Patient A showed a dramatic difference in this parameter pre and post foscarnet. Nevertheless, history of the patient such as unexplained and serious fall in academic or work performance could corroborate brain wave abnormalities and suggest reactivation. Zerr et al. showed that HHV-6 reactivation in HSCT patients is significantly associated with neurocognitive decline in executive functioning, attention, processing speed and concentration.26

Since valganciclovir carries a risk of bone marrow suppression, patients must be regularly monitored for severe drops in white blood cell numbers during treatment. Neutropenia can be resolved through proper dosage adjustment.28 Foscarnet must be administered via PICC line and the patient must be monitored for kidney toxicity, as foscarnet administration carries the risk of renal toxicity, especially if the patient is not properly hydrated.29 The family was informed that ganciclovir has caused infertility and cancer in animal studies.

Some have questioned whether CIHHV-6 is associated with actively replicating virus and symptoms.16,30 In addition to the clinical responses to antiviral therapy described and reviewed here, work by Hall et al. on mother–infant pairs suggests that CIHHV-6 can reactivate in vivo.31 Maternal and offspring HHV-6 variants
matched, suggesting that integrated latent virus of the mother reactivated and infected the infant in utero.  

In 194 children and infants evaluated for encephalitis, there was a CIHHV-6 prevalence of 3.3%, or four times the rate in normal controls, raising the possibility that reactivation of CIHHV-6 may be the trigger of encephalitis in some patients. 

Several case reports have suggested reactivated virus in CIHHV-6 patients. In contrast, several case reports have described CIHHV-6 patients or recipients of CIHHV-6 donor cells in HSCT without apparent reactivation in spite of significant immunosuppression. It is possible that in some cases of CIHHV-6 the virus is defective and unable to reactivate; for example, the integrated viral genome has suffered a mutation in one of the viral genes essential for lytic replication. 

Lee et al. showed that CIHHV-6 status in liver transplants is associated with an increased rate of allograft rejections and opportunistic infections and Potenza et al. described a case of a CIHHV-6 patient who received an allogenic stem-cell transplantation. The patient was treated successfully with antivirals twice when the leukopenia coincided with an increased plasma load of HHV-6 DNA. A bone marrow biopsy using HHV-6A early protein (gp41/38) indicated productive infection of HHV-6A. 

Tanaka-Taya et al. found that late HHV-6 antibody titers of CIHHV-6 patients were lower than those of healthy volunteers, perhaps due to tolerance. In this case report, HHV-6 IgG antibodies were only modestly elevated (1:640) at the start of treatment and fell by 2 (Patient A) and 4 (Patient B) dilutions over time (Fig. 1). 

Since CIHHV-6 patients may be unable to mount effective immune responses due to tolerance, it is possible that their symptoms are due to exogenous HHV-6 strains. This possibility can be addressed in the future by comparing the nucleotide sequence of HHV-6 mRNA and the inherited integrated viral genome. 

What may trigger symptomatic activation of CIHHV-6 also remains unknown. Both patients in this report were found to have mild hypogammaglobulinemia during the investigations of their symptoms. Transient hypogammaglobulinemia may precede HHV-6 reactivation, and hypogammaglobulinemia has been linked to HHV-6 reactivation in patients with Drug Induced Hypersensitivity Syndrome (DIHS), a condition now recognized as a complex interaction that may involve HHV-6. 

Both siblings suffered from asthma and were on a steroid inhaler for asthma. Hydrocortisone is known to activate HHV-6 replication in vitro, and a Japanese study suggests that steroid administration is associated with an increased risk of HHV-6 encephalitis in hematopoietic stem cell transplantation (HSCT) patients. 

Patient A had elevated serum IL-6 on two occasions. Serum and CSF levels of IL-6 have been associated with HHV-6 encephalopathy. IL-6 levels may be important for predicting neurological sequelae of HHV-6 infection, but it is not clear whether this is a cause or an effect. Patient B was not tested for serum IL-6. Typical features of HHV-6 encephalitis include diffuse slowing with focal spikes and both patients showed abnormalities on qEEG consistent with HHV-6 encephalopathy. 

This report demonstrates that CIHHV-6 patients presenting with chronic or acute CNS dysfunction might benefit from antiviral therapy. This finding supports the emerging hypothesis that in some cells of CIHHV-6 patients the virus reactivates, or can become infected by an exogenous HHV-6 strain. In either case, abundant late proteins associated with viral replication are produced; the reactivated cells either die because of lytic virus replication, or they are killed by immune responses resulting in a chronic state of inflammation. These events may lead to clinical symptoms. Further work and novel assays are needed to distinguish between CIHHV-6 integrants that can reactivate and those that are defective. Tolerance and increased susceptibility to community-acquired strains may be important factors to consider in these patients.

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**Competing interests**

All authors have no competing interest.

**Ethical approval**

The study was approved by the Institutional Review Board.

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**Appendix A. Supplementary data**

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcv.2012.05.016.

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