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A fatal case of JC virus meningitis presenting with hydrocephalus in an HIV-seronegative patient

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Abstract

JC virus (JCV) is the etiologic agent of progressive multifocal leukoencephalopathy, JCV granule cell neuronopathy and JCV encephalopathy. Whether JCV can also cause meningitis, has not yet been demonstrated. We report a case of aseptic meningitis resulting in symptomatic hydrocephalus in an HIV-seronegative patient. Brain imaging showed enlargement of ventricles but no parenchymal lesion. She had a very high JC viral load in the CSF and developed progressive cognitive dysfunction despite ventricular drainage. She was diagnosed with pancytopenia and passed away after 5 ½ months. Post-mortem exam revealed productive JCV infection of leptomeningeal and choroid plexus cells, and limited parenchymal involvement. Sequencing of JCV CSF strain showed an archetype-like regulatory region. Further studies of the role of JCV in aseptic meningitis and in idiopathic hydrocephalus are warranted.

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INTRODUCTION

JC virus (JCV) is a ubiquitous human polyomavirus which infects healthy people without causing any diseases. In the setting of immunosuppression, this virus can reactivate and destroy oligodendrocytes and astrocytes, leading to demyelinating disease of the brain called progressive multifocal leukoencephalopathy (PML). JC virus variants can also cause productive infection of cerebellar granule cell or cortical pyramidal neurons, resulting in JCV granule cell neuronopathy (JCV GCN) or JCV encephalopathy (JCVE), respectively.[1] However, whether JCV is also associated with meningitis remains unclear. JC virus DNA was detected by PCR in 2 of 131 CSF samples from patients with suspected meningitis or encephalitis[2]. None of them had parenchymal brain lesions. There are only 2 case reports of JC virus meningitis. One concerned an immunocompetent girl, who was diagnosed based on rising titers of JCV antibodies in the serum, leading to a hypothesis that this was a primary infection. The presence of the virus was not demonstrated in the CSF[3]. The second described a woman with systemic lupus erythematosus, presenting with fever, headaches, and altered consciousness, who had a positive JCV PCR in her CSF. MRI showed mild ventricular dilatation without any white matter lesions. She recovered spontaneously and the diagnosis was not confirmed histologically[4].

Here, we report a case of JCV-associated meningitis presenting with hydrocephalus, in an HIV-seronegative patient. We also describe histologic findings and analyses of the viral strain isolated from her CSF.

CASE REPORT

A 67 year old, previously healthy female, presented with new onset, progressively worsening headache associated with vomiting. Over the next 2 months, she developed urinary incontinence and weakness in her legs. She became increasingly forgetful and lethargic to the point where she could not recognize her family members, leading to hospital admission. She had a past medical history of uterine myomectomy, and was under treatment for high blood pressure. Over the 2 years prior to her admission, she had been treated for recurrent urinary tract infections, thrush, bronchitis and community acquired pneumonia. She had just completed a two weeks course of dimercaptosuccinic acid (DMSA) chelation therapy for mercury and iron toxicity based on a lab test that was later deemed inaccurate by the local poison control department. Her only medication at the time of hospitalization was telmisartan. She had emigrated from Malta at the age of 12 and lived in the northeastern United States since then, where she worked for a family business. She had been on a cruise to Caribbean islands after the onset of her headache. She did not use tobacco or illicit drugs and drank alcohol only occasionally. Her family history was noncontributory.

On examination, she was afebrile with a heart rate of 76 beats per minute and blood pressure of 122/76 mm Hg. Her oxygen saturation was 99% on room air. She was alert but confused. Motor strength, deep tendon reflexes and sensory exam were normal. She demonstrated gait apraxia.

Laboratory tests showed a normal complete blood count, electrolytes and liver function tests. Initial head CT revealed enlargement of all ventricles which was confirmed on the

MRI (Figure 1). A lumbar drain was placed due to a concern for normal pressure hydrocephalus. The opening cerebrospinal fluid (CSF) pressure was 30 cm H_2O. CSF analysis showed 10 white blood cells per cubic millimeter with 92% lymphocytes and protein concentration of 61 mg/dl. Given improvements in her cognition after the lumbar drain, a right ventriculo-peritoneal shunt (VPS) was placed. Although her cognitive function initially improved, she required continued assistance at a rehabilitation facility and was readmitted within 3 weeks due to worsening headaches and cognition. There was no VPS malfunction. Whole body CT and PET scans were normal. A comprehensive work-up (Table 1) failed to reveal an etiology and her cognitive function deteriorated further.

A brain biopsy, performed 4 months after symptoms onset, that included the right middle meningeal artery, frontal dura matter, frontal lobe brain parenchyma and periventricular region, showed astrogliosis with foci of histiocytic infiltration and modest perivascular cuffing by T lymphocytes. Specific staining for cytomegalovirus and herpes simplex virus -1 and 2 were negative. She received dexamethasone 6mg IV every 6 hours for 5 days and then tapered over 4 days. Eventually, CSF JCV PCR was performed, which showed 8.9 million copies of JCV DNA per ml. The results of multiple CSF analyses and blood tests during her hospitalizations are shown in Table 1. She was seronegative for HIV 1 and 2 but developed lymphocytopenia with reduced T and B cell counts and immunoglobulin levels (Table 1). She was started on mirtazapine 15mg at bedtime and received intravenous immunoglobulin for 3 days. She was intubated for respiratory failure in the setting of hospital-acquired pneumonia. She continued to decline neurologically and life support was withdrawn. She passed away 5 and a half months after the onset of symptoms.

METHODS

Clinical specimens

At autopsy, the whole brain was fixed in 10% neutral buffered formalin for 2 weeks and tissue blocks were paraffin embedded. Single and double immunohistochemistry (IHC) stainings for JCV large T antigen (T Ag) and major capsid protein VP1 were done as previously described using ImmPRESS Polymer Detection Reagents (Vector laboratories, Burlingame, CA) following the manufacturers recommendations.[5] For some of the experiments, a Luxol Fast Blue counter-staining was performed to determine the extent of demyelination.

PCR and sequence analysis

Quantified PCR for JCV DNA was performed in CSF using JCV specific primers as previously described[6]. The JCV regulatory region and Agno gene were amplified with primer pair CPN1Ag45 and CPN1Ag43[7], while the VP1 C terminus gene was amplified with primer pair CJS2465 and CJR2578, as described previously[8]. All PCR products were cloned into pSC-B-amp/kan vector using StrataClone Blunt PCR Cloning Kit (Agilent, Santa Clara, CA). Positive clones were selected and sequenced using an ABI 3700 apparatus.

JCV Antibody analysis

IgM, IgA and IgG antibodies against JCV were detected in the patient's plasma using a virus-like particle-based enzyme-linked immunosorbent assay, as described previously[9]. JCV antibody index was measured in plasma using a commercial assay (Focus Diagnostics, Cypress, CA)

Cellular immune response to JCV

At 4.5 months after symptom onset, we performed intracellular cytokine staining (ICS) for detection of JCV-specific T cells in the patient's blood, as described previously[10].

RESULTS

Post mortem examination demonstrated thin and translucent leptomeninges. The lateral ventricles were enlarged. There were no white matter lesions on macroscopic examination. Hematoxilin & eosin staining demonstrated focal areas of viral cytopathic changes in the periventricular white matter including chromatin margination and nuclear enlargement in oligodendrocytes and reactive astrocytes. Myelin stain showed limited areas of focal demyelination in the periventricular white matter but none in the subcortical or cerebellar white matter. IHC staining demonstrated significant JCV infection of the leptomeningeal cells of the pia surrounding the cerebrum and cerebellum, which expressed both JCV T Ag and VP1 capsid protein (Fig 2A). JCV-infected cells were also present in the wall of meningeal arteries and in the subpial brain parenchyma (Fig 2. A and B). In the cerebellum, JCV-infected subpial cells were detected in immediate contact with projecting fibers of cells from the granule cell layer (Fig 2C). In addition, isolated granule cell neurons also expressed JCV proteins, but the cerebellar white matter was devoid of infected cells. Finally, JCV infection also involved epithelial cells of choroid plexus (Fig 2D). While JCV T Ag indicates early or restrictive infection, JCV VP1 capsid protein expression signifies the presence of mature viral particles. Compared to the total number of JCV-infected cells, a productive infection, demonstrated by single IHC staining for VP1, was detected in 10% of choroid plexus cells, 80% of leptomeningeal cells, 90% of cerebellar subpial molecular layer cells, and 10% cells of cerebellar granule cell layer infected by JCV (data not shown). There was moderate parenchymal infiltration by $CD3^+$ T-cells but very few $CD8^+$ T-cells (data not shown).

The sequence of the JCV strain isolated from the CSF, named JCV_{M1}, showed an archetype-like regulatory region (RR) bearing a small deletion in the 66 bp insert in 9/10 clones (Fig 3). A minority RR (1/10 clones) with deletion of nt 37-209 was also observed. There was no DNA mutation in the agno gene. A total of 4 silent mutations were found in the VP1 capsid gene C-terminus. A blood sample collected 4.5 months after onset of symptoms showed detectable anti JCV IgG, but no IgM or IgA antibodies. The JCV antibody index was positive at 1.78. ICS showed no $CD8^+$ T-cell response and minimal $CD4^+$ T-cell response to JCV VP1 peptides.

DISCUSSION

This is the first detailed description of JCV meningitis (JCVM), as demonstrated by an extremely high CSF viral load, up to 48 million copies of JCV DNA/ml, and productive JCV infection of leptomeningeal cells. The causality of JCV in this condition is supported by sequence-based criteria for identification of microbial pathogens[11]. This initial presentation is very different from the known clinical syndromes of PML, JCV-GCN and JCVE. The patient developed subacute meningitis leading to hydrocephalus, with the associated triad of cognitive, gait and urinary dysfunction. This is quite distinct from the focal motor or sensory deficits observed in PML[12], the cerebellar ataxia of JCV GCN[13, 14] and the fulminant encephalopathy of JCVE[15]. Moreover, imaging studies did not show any white matter lesions, cerebellar atrophy or cortical abnormalities, leading to a delay in diagnosis. In absence of other explanation, the patient most likely developed communicating hydrocephalus due to poor CSF reabsorption from the infected meninges. Arachnoid villi are disrupted by the removal of the brain from the skull during the post-mortem examination, and therefore could not be examined histologically. While there was no contrast-enhancement on MRI, hyperintense signal was detected on FLAIR images in the sulci. This finding can be attributed to high protein and cellular concentration in the CSF[16]. The presence of millions of copies of JCV DNA in the CSF is also atypical for PML, where the median CSF viral load is in thousands[17]. Interestingly, some of the epithelial cells from the choroid plexus showed productive JCV infection, which suggest that these cells contributed to the very high JCV burden in CSF.

The brain showed predominant JCV infection of the leptomeninges, the presence of infected subpial and juxtaventricular cells, and relative paucity of infected cells deeper in the brain parenchyma and demyelination. This unique pattern suggest a route of infection from the CSF towards the brain, rather than the opposite as seen in the other JCV-associated brain diseases, where the infection initiates in the parenchyma, and JCV is only later detected in the CSF which remains devoid of inflammation. Overtime, this patient developed microscopic parenchymal JCV infection in the cerebral and cerebellar cortex and periventricular areas, consistent with a meningoencephalitis, but this was not apparent on MRI. Infection of leptomeningeal cells was productive, with expression of both JCV T Ag, present early in the viral cycle, and the late capsid protein VP1. Conversely, most infected cells located in the cerebrum or cerebellum away from the CSF spaces, expressed the T Ag only, consistent with an early or restrictive JCV infection. Her humoral immune response to JCV was suggestive of a viral reactivation rather than a primary infection. Her cellular immune response to JCV was weak and a limited inflammatory infiltrate was present near JCV-infected brain and meningeal cells.

The JCV RR contains determinants of neurotropism and neurovirulence. While the archetype RR is considered non-pathogenic and is commonly found in the kidney and urine samples of healthy and immunosuppressed individual alike, rearranged RR with duplications of the 98 bp elements are usually found in the CNS of PML or JCV GCN patients[8, 18]. However, we recently reported an archetype-like RR in a patient with JCVE, indicating that this form could also be pathogenic in the CNS[7]. This is confirmed by the isolation of another archetype-like RR in the CSF of our present patient with JCVM. Targeted molecular

analyses demonstrated that the CSF JCV strain did not have the agno or VP1 gene mutations found in JCVE or JCV GCN cases[19]. Further studies, now in progress in our laboratory, will be necessary to determine whether the JCVM strain harbors mutations in different sites of the viral genome.

The exact etiology of lymphocytopenia, affecting both T and B cell lineages in this patient could not be established. The role of DMSA in causing bone marrow suppression was considered, but lymphocytopenia is not a known side effect of DMSA[20]. She only developed lymphocytopenia three months into her illness, although she had recurrent infections for 2 years, suggesting an underlying immunosuppression. Unfortunately, bone marrow was not available for analysis either ante or post-mortem.

This case establishes JCV as an etiologic agent of meningitis. Further studies to elucidate the prevalence of JCV DNA in CSF of patients with aseptic meningitis and those presenting with idiopathic hydrocephalus are warranted.

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References

1. Gheuens S, Wuthrich C, Koralnik IJ. Progressive multifocal leukoencephalopathy: why gray and white matter. Annu Rev Pathol. 2013 Jan 24.8:189–215. [PubMed: 23092189]
2. Behzad-Behbahani A, Klapper PE, Vallely PJ, Cleator GM, Bonington A. BKV-DNA and JCV-DNA in CSF of patients with suspected meningitis or encephalitis. Infection. 2003 Dec; 31(6):374–8. [PubMed: 14735377]
3. Blake K, Pillay D, Knowles W, Brown DW, Griffiths PD, Taylor B. JC virus associated meningoencephalitis in an immunocompetent girl. Arch Dis Child. 1992 Jul; 67(7):956–7. [PubMed: 1325756]
4. Viallard JF, Ellie E, Lazaro E, Lafon ME, Pellegrin JL. JC virus meningitis in a patient with systemic lupus erythematosus. Lupus. 2005; 14(12):964–6. [PubMed: 16425577]
5. Wuthrich C, Koralnik IJ. Frequent infection of cortical neurons by JC virus in patients with progressive multifocal leukoencephalopathy. J Neuropathol Exp Neurol. 2012 Jan; 71(1):54–65. [PubMed: 22157619]
6. Ryschkewitsch C, Jensen P, Hou J, Fahle G, Fischer S, Major EO. Comparison of PCR-southern hybridization and quantitative real-time PCR for the detection of JC and BK viral nucleotide sequences in urine and cerebrospinal fluid. J Virol Methods. 2004 Nov; 121(2):217–21. [PubMed: 15381359]
7. Dang X, Wuthrich C, Gordon J, Sawa H, Koralnik IJ. JC virus encephalopathy is associated with a novel agnoprotein-deletion JCV variant. PLoS One. 2012; 7(4):e35793. [PubMed: 22536439]
8. Dang X, Koralnik IJ. A granule cell neuron-associated JC virus variant has a unique deletion in the VP1 gene. J Gen Virol. 2006 Sep; 87(Pt 9):2533–7. [PubMed: 16894191]
9. Viscidi RP, Khanna N, Tan CS, et al. JC virus antibody and viremia as predictors of progressive multifocal leukoencephalopathy in human immunodeficiency virus-1-infected individuals. Clin Infect Dis. 2011 Oct; 53(7):711–5. [PubMed: 21852452]
10. Gheuens S, Bord E, Kesari S, et al. Role of CD4+ and CD8+ T-cell responses against JC virus in the outcome of patients with progressive multifocal leukoencephalopathy (PML) and PML with immune reconstitution inflammatory syndrome. J Virol. 2011 Jul; 85(14):7256–63. [PubMed: 21543472]

11. Fredericks DN, Relman DA. Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. Clin Microbiol Rev. 1996 Jan; 9(1):18–33. [PubMed: 8665474]
12. Berger JR, Aksamit AJ, Clifford DB, et al. PML diagnostic criteria: consensus statement from the AAN Neuroinfectious Disease Section. Neurology. 2013 Apr 9; 80(15):1430–8. [PubMed: 23568998]
13. Schippling S, Kempf C, Buchele F, et al. JC virus granule cell neuronopathy and GCN-IRIS under natalizumab treatment. Ann Neurol. 2013 Jul 19.
14. Shin HW, Kang SY, Sohn YH. JC viral infection-related cerebellar degeneration as the first manifestation of AIDS. Eur Neurol. 2008; 59(3–4):205–7. [PubMed: 18230885]
15. Wuthrich C, Dang X, Westmoreland S, et al. Fulminant JC virus encephalopathy with productive infection of cortical pyramidal neurons. Ann Neurol. 2009 Jun; 65(6):742–8. [PubMed: 19557867]
16. Stuckey SL, Goh TD, Heffernan T, Rowan D. Hyperintensity in the subarachnoid space on FLAIR MRI. AJR Am J Roentgenol. 2007 Oct; 189(4):913–21. [PubMed: 17885065]
17. Bossolasco S, Calori G, Moretti F, et al. Prognostic significance of JC virus DNA levels in cerebrospinal fluid of patients with HIV-associated progressive multifocal leukoencephalopathy. Clin Infect Dis. 2005 Mar 1; 40(5):738–44. [PubMed: 15714422]
18. Jensen PN, Major EO. A classification scheme for human polyomavirus JCV variants based on the nucleotide sequence of the noncoding regulatory region. J Neurovirol. 2001 Aug; 7(4):280–7. [PubMed: 11517403]
19. Dang X, Koralnik IJ. Gone over to the dark side: Natalizumab-associated JC virus infection of neurons in cerebellar gray matter. Ann Neurol. 2013 Aug 2.
20. Bradberry S, Vale A. Dimercaptosuccinic acid (succimer; DMSA) in inorganic lead poisoning. Clin Toxicol (Phila). 2009 Aug; 47(7):617–31. [PubMed: 19663612]

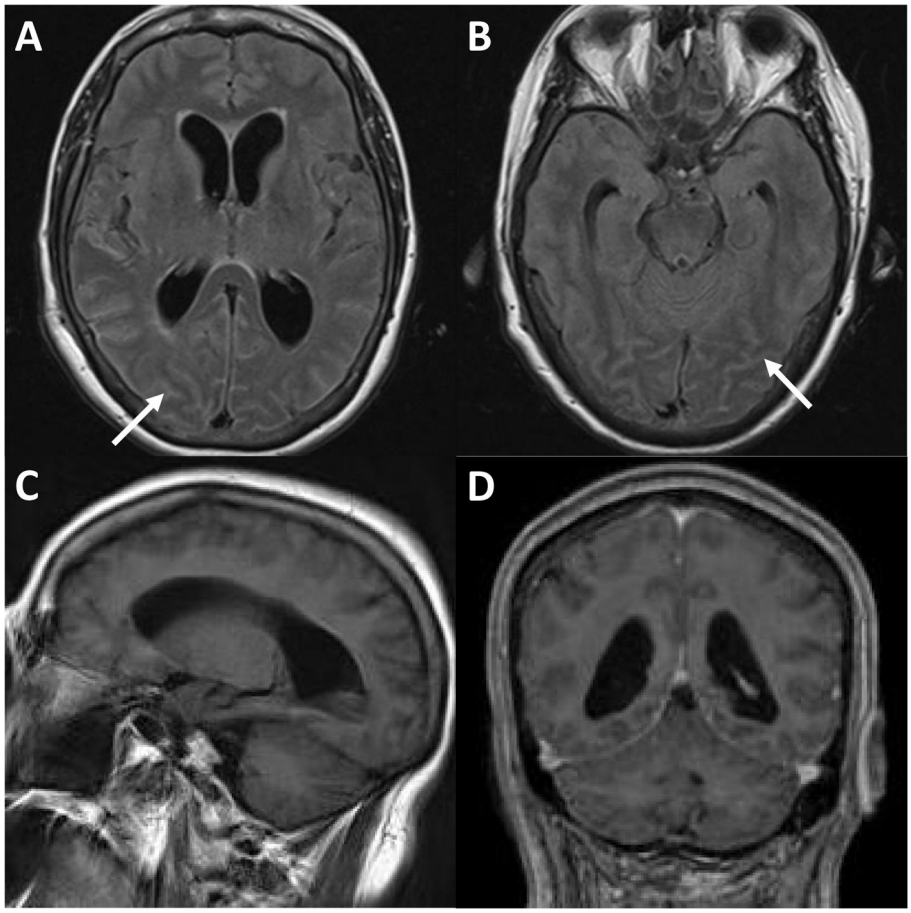

Figure 1. Magnetic Resonance Imaging of the brain demonstrates hydrocephalus and abnormal signal in subarachnoid space

MRI performed 2.5 months after symptoms onset shows hydrocephalus and no parenchymal lesions. Panels A and B are axial FLAIR sequences showing enlarged ventricles and abnormal hyperintensity in the subarachnoid space, within the sulci of the cerebral hemispheres (arrows). Panel C is a sagittal T1-weighted sequence demonstrating significant enlargement of the lateral ventricle. In Panel D, there was no enhancement of the meninges post intravenous contrast administration, as seen on this coronal section.

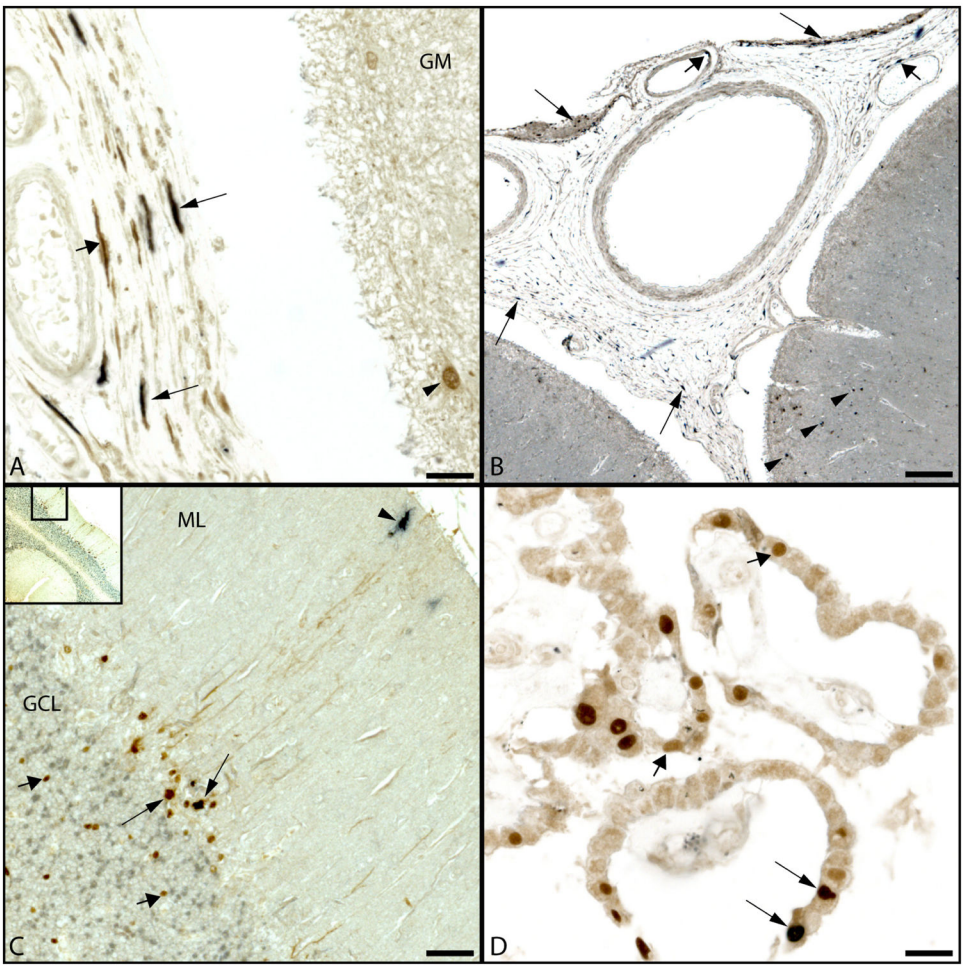

Figure 2. Immunohistochemistry (IHC) analyses of JCV infection in the CNS

Panel A shows double IHC staining for JCV T Ag (brown) and JCV VP1 protein (blue). Numerous JCV-infected leptomeningeal cells expressing VP1 protein (long arrow) or T Ag (short arrow) are overlying the cerebral cortex. A JCV-infected cell can be seen in the subpial gray matter (GM) of the cerebral cortex, expressing T Ag (arrowhead) Bar: 25um. Panel B shows a lower magnification of the cerebral cortex, which reveals many JCV-infected cells in the leptomeninges (long arrows) as well as in the walls of meningeal vessels (short arrows), and few JCV infected cells in the cortical GM (arrowheads). Bar: 200um. Panel C shows a high magnification of the cerebellum (boxed area in the inset), with a subpial cell in the molecular layer (ML) expressing JCV VP1 protein (arrowhead) close to projecting fibers of cells from the granule cell layer (GCL) expressing JCV T Ag (long arrows). Isolated JCV-infected cells, expressing T Ag can be seen in the GCL (short arrows). Bar: 50um. Panel D shows JCV-infected epithelial cells of the choroid plexus of the lateral ventricles expressing VP1 protein (long arrows) or T Ag (short arrows). Bar: 25um.

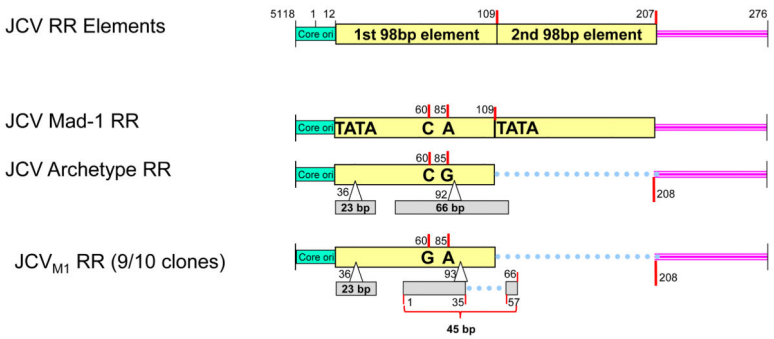

Figure 3. Comparison of JCV regulatory regions (RR)

The prototype CNS strain JCV$_{Mad-1}$ has two identical 98 bp elements, whereas the archetype RR, commonly found in kidneys and urine samples has a single 98 bp element and a 23 bp and 66 bp inserts. The JCV$_{M1}$ RR from the CSF of the JCV meningitis patient has an archetype-like RR with two single nucleotide mutations in the 98 bp element and partial deletion of the 66 bp insert. This insert is located at nt position 93, instead of nt position 92 in the archetype RR.

TABLE 1

Cerebrospinal fluid and blood laboratory results

Variable	Reference range, Adults	First Admission	Second admission	Hospital Day 42
CEREBROSPINAL FLUID				
Source of CSF		Spinal tap	Shunt	Shunt
Red blood cell count (per mm3)	0	244	5280	402
White blood cell count (per mm3)	0–5	10	4	3
Protein (mg/dl)	5–55	61	169	187
Glucose (mg/dl)	50–75	50	72	75
Gram stain	No bacteria	No bacteria	No bacteria	No bacteria
Bacterial and fungal culture	No growth	No growth	No growth	No growth
VDRL	Non-reactive	Non-reactive		
Cytology	No abnormal cells	No abnormal cells		No abnormal cells
Protein 14-3-3	<1.0		<1.0	
Listeria Ab (titer)	<1:8		<1:8	
ACE level (ACE units)	<4		<4	
Neuron specific enolase (ng/mL)	<15		<5.0	
HSV I and II PCR	Negative	Negative	Negative	Negative
Coccidiodes antibody, IgM and IgG	Negative		Negative	
VZV PCR	Negative	Negative		Negative
EBV PCR	Negative			Negative
Enterovirus PCR	Negative			Negative
West Nile Virus PCR	Negative	Negative		Negative
JCV PCR (copies/ml)	Negative		8.9×10^6	48.2×10^6
anti-GQ1b IgG (titer)	<1:100		<1:100	
Paraneoplastic panel*	Negative		Negative	
Oligoclonal bands on gel electrophoresis	None in CSF		10 Oligoclonal bands matched to serum	
BLOOD				
White cell count (per mm^3)	4000 – 11000	5200	7500	3900
Absolute Lymphocyte counts (per mm3)	700–3500	1057	267	251
$CD3^+$ T-cell count (per mm3)	578–1850		168	
$CD4^+$ T-cell count (per mm3)	350–1100		32	120
$CD19^+$ B-cell count (per mm3)	169–271		0	
Serum Immunoglobulin G (mg/dl)	700–1500		223	456
Serum Immunoglobulin M (mg/dl)	60–300		66	45
Serum Immunoglobulin A (mg/dl)	60–400		55	54
Lyme serology	Negative	Negative		
HIV serology	Negative		Negative	Negative
HIV viral load	Negative		Negative	Negative

Variable	Reference range, Adults	First Admission	Second admission	Hospital Day 42
Anti-nuclear antibodies	Negative		Negative	
Sjogren's antibodies (anti-Ro, anti-La)	Negative		Negative	
Rheumatic factor	Negative		Negative	
Anti-neutrophil cytoplasmic antibody	Negative		Negative	
Anti-cardiolipin antibodies	Negative		Negative	

* Includes anti-neuronal nuclear antibodies, anti-glial nuclear antibodies, purkinje cell cytoplasmic antibodies, amphiphysin antibody, CRMP-5-IgG, striated muscle antibody, P/Q-type calcium channel antibody, N-Type calcium channel antibody, ACh receptor (muscle) binding antibody, AChR ganglionic neuronal antibody, neuronal (V-G) K+ channel antibody