JCV GCN in a natalizumab-treated MS patient is associated with mutations of the *VP1* capsid gene

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ABSTRACT

Objective: To describe the clinical, neuroimaging, immunologic, and virologic characteristics of JC virus–associated granule cell neuronopathy (JCV GCN) in a natalizumab-treated patient with multiple sclerosis (MS) who developed immune reconstitution inflammatory syndrome (IRIS) after natalizumab withdrawal.

Methods: We obtained longitudinal clinical data as well as MRI and proton magnetic resonance spectroscopy from this patient with MS. We measured JCV-specific cellular immune response in his peripheral blood by intracellular cytokine staining and sequenced a fragment of JCV *VP1* capsid gene detected in his CSF. We contrast our findings with the first recently reported case.

Results: This patient presented with worsening cerebellar symptoms and progressive cerebellar atrophy without new MS lesions on MRI after 63 months of natalizumab monotherapy. JCV DNA was detected in his CSF by PCR and harbored novel GCN-type mutations in the *VP1* gene. He developed IRIS upon discontinuation of natalizumab and plasma exchange, which manifested itself by a worsening of clinical symptoms and contrast enhancement in the cerebellum on MRI. Treatment with corticosteroids resulted in resolution of IRIS, as demonstrated by proton magnetic resonance spectroscopy. The patient had a strong JCV-specific T-cell response in his peripheral blood and remains alive after 15 months from onset of symptoms, although with significant disability. He did not have MS relapse on glatiramer acetate.

Conclusions: JCV GCN should be considered in patients on natalizumab presenting with progressive cerebellar symptoms and cerebellar atrophy, and is associated with mutations in the JCV *VP1* gene. Natalizumab withdrawal may be complicated by JCV GCN IRIS, and require treatment with corticosteroids. *Neurology*® 2014;83:727–732

GLOSSARY

aa = amino acid; **bp** = base pair; **GCN** = granule cell neuronopathy; **^{1}H-MRS** = proton magnetic resonance spectroscopy; **IRIS** = immune reconstitution inflammatory syndrome; **JCV** = JC virus; **MS** = multiple sclerosis; **PLEX** = plasma exchange; **PML** = progressive multifocal leukoencephalopathy; **RR** = regulatory region.

JC virus (JCV) causes lytic infection of oligodendrocytes, leading to demyelinating disease of the brain called progressive multifocal leukoencephalopathy (PML). In addition, JCV may infect cerebellar granule cell neurons resulting in JCV granule cell neuronopathy (GCN).[1]

JCV GCN, described in patients with HIV, sarcoidosis, and CD40 ligand deficiency, is associated with mutations in the C terminus of the JCV *VP1* gene, coding for the major capsid protein.[2,3] Natalizumab, which prevents egress of lymphocytes from the bloodstream, is used for treatment of multiple sclerosis (MS) and Crohn disease. As of March 6, 2014, 448 patients with MS and 2 patients with Crohn disease treated with natalizumab have developed PML worldwide.[4] Recently, JCV GCN was also described in a patient with MS presenting with cerebellar symptoms and atrophy after 52 months of natalizumab monotherapy.[5] This patient developed immune reconstitution inflammatory syndrome (IRIS) after natalizumab withdrawal, characterized by inflammatory infiltrates in the cerebellar parenchyma, but did not have contrast enhancement on MRI. Whether JCV GCN caused by natalizumab is also associated with

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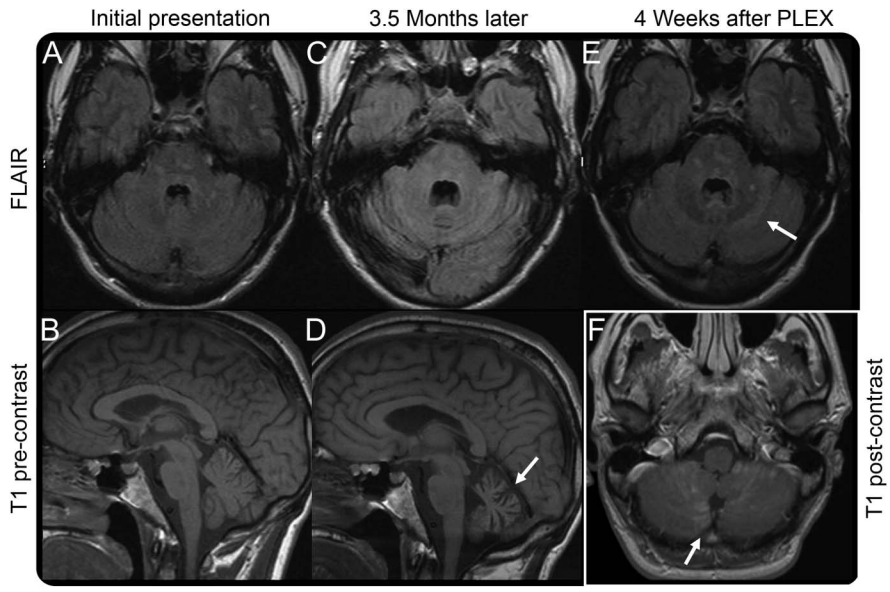
JCV *VP1* mutations is unknown. We present here the second case of natalizumab-associated JCV GCN. Sequential MRI studies, results of proton magnetic resonance spectroscopy (^{1}H-MRS), and testing of JCV-specific immune response highlight differences with the initial case. Sequence analysis revealed novel GCN-type mutations in the *VP1* gene.

CASE A 32-year-old man with MS for 12 years was switched to natalizumab from interferon β-1b, when he experienced repeated relapses. He was JCV-seropositive and had received 63 infusions of natalizumab monotherapy when he presented with changes in his handwriting, speech, and gait. MRI, done 2 weeks after onset of symptoms (figure 1, A and B), showed no new lesions. He was treated with oral corticosteroids for suspected MS exacerbation, but continued to have progressive cerebellar symptoms. CSF analysis 3 months after onset of symptoms showed 2 white blood cells/mm^3 with a protein concentration of 70 mg/dL. JCV PCR was positive, confirming the diagnosis of JCV GCN. Natalizumab was discontinued and he received plasma exchange (PLEX) for 5 days, followed by mirtazapine 15 mg/d orally. IV methylprednisolone was given for 5 days to prevent IRIS. MRI showed progressive cerebellar atrophy without any confluent white matter lesions (figure 1, C and D). Approximately 4 weeks after PLEX, he developed vertigo, headaches, and dysphonia, and CSF examination showed 71 white blood cells/mm^3 with 95% lymphocytes and protein of 136 mg/dL. CSF JC viral load was 16,489 copies/mL. There were hyperintense lesions in the pons and cerebellar peduncles on fluid-attenuated inversion recovery MRIs, as well as contrast enhancement within the cerebellum (figure 1, E and F), suggestive of IRIS. He was treated with 7 days of IV methylprednisolone and was started on maraviroc given a putative benefit in IRIS, while remaining on mirtazapine.

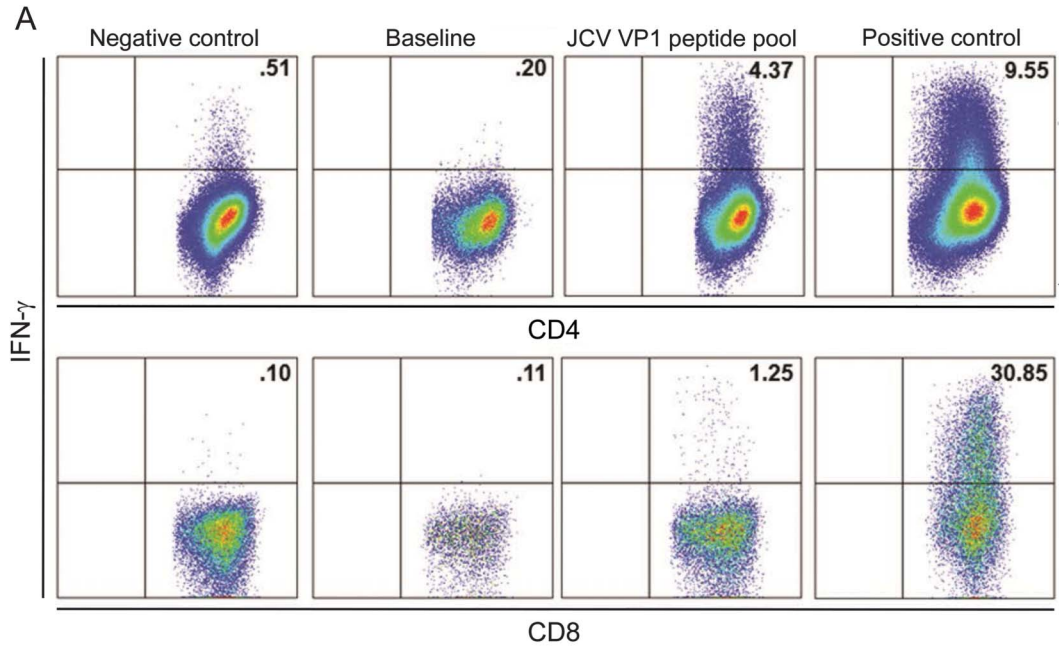
Intracellular cytokine staining, performed as described previously, showed a robust cellular immune response mediated by JCV-specific CD4$^+$ and CD8$^+$ T cells producing interferon γ (figure 2A).[6] Molecular analysis of CSF JCV strains, performed as previously described, revealed mutations in the C terminus of the *VP1* gene, consistent with GCN-type JCV strains (figure 2B).[3] A total of 3 new GCN-type JCV strains were found: JCV_{GCN1b} (16.7% clones) has identical 10–base pair (bp) deletion as reported for JCV_{GCN1} in a different backbone; JCV_{GCN6} (66.7% clones) has a novel 3-bp deletion between nt 2,497 and 2,499; and JCV_{GCN7} (16.7% clones) has a novel 18-bp deletion between nt 2,494

Figure 1 **MRIs of cerebellum and brainstem demonstrate progressive cerebellar atrophy and immune reconstitution inflammatory syndrome**



(A) FLAIR and (B) precontrast T1 images are taken within 2 weeks of symptoms onset. (A) Few hyperintense lesions in the cerebellar peduncles that had been stable for many years. (B) Baseline size of the cerebellum. (C) FLAIR and (D) precontrast T1 images done 4 months after initial presentation and a week after positive CSF JC virus PCR show marked cerebellar atrophy compared with the scan done 3.5 months before (B) without any new hyperintense lesions in the cerebellar peduncles. FLAIR (E) and postcontrast T1 (F) images done 4 weeks after plasma exchange treatment identify new diffuse hyperintensities in the cerebellum as well as contrast enhancement, suggestive of immune reconstitution inflammatory syndrome. FLAIR = fluid-attenuated inversion recovery; PLEX = plasma exchange.

Figure 2 Strong T-cell response to JCV and mutations in C terminus of JCV *VP1* gene



B

DNA sequence:

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NT #  2471                                  2503     2512                  2533
JCV_Mad1   GGGGACCCAGACATGATGAGATACGTTGACAAATATGGACAGTTGCAGACAAAAATGCTGTAA
JCV_GCN1   --A--------T----------T-------G          ----------------------
JCV_GCN1b  --A--------T------------------G          ----------------------
JCV_GCN4   --A--------T----------T-------G          A-ATG------G---------
JCV_GCN2   --A--------T----------T-------G--------      -A-----G---------
JCV_GCN3   --A--------T--GATGAGATATGTTGACAG----------------------G---------
           17 bp Insertion: GATGAGATATGTTGACA
JCV_GCN5   --A--------T----------T-           -------------------------
JCV_GCN6   --A--------T-------------   --G-------------------------------
JCV_GCN7   --A--------T----------                 ----------------------
                                2494          2511                  2533
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aa sequence (N–C):

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aa #: 335       345      354
JCV_Mad1   GDPDMMRYVDKYGQLQTKML.
JCV_GCN1   GDPDMMRYVDS  CRQKCCNQKPLL.
JCV_GCN4   GDPDMMRYVDR  YGQRCCNQKPLL.
JCV_GCN2   GDPDMMRYVDKYG  QTKML.
JCV_GCN3   GDPDMMRYVDR.
JCV_GCN5   GDPDMMRY    GQLQTKML.
JCV_GCN6   GDPDMMRYV RYGQLQTKML.
JCV_GCN7   GDPDMMR.
           341
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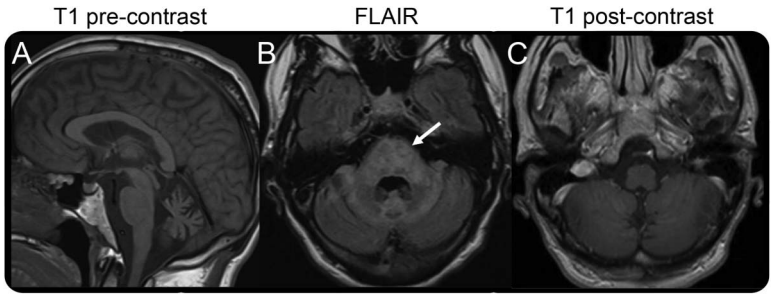
Type	Underlying condition	Origin
GCN1	HIV & MS NTZ	Boston & Brazil & Scottsdale
GCN2	HIV	New York
GCN3	CD40L	San Francisco
GCN4	HIV	Brazil
GCN5	NHL/Rituximab	Ann Arbor
GCN6, 7	MS NTZ	Scottsdale

(A) Strong interferon (IFN) γ response is observed in $CD4^+$ and $CD8^+$ T cells from peripheral blood after stimulation with JCV peptides 7 months after initial presentation. The percentages of IFNγ-expressing $CD4^+$ T cells (upper row) and $CD8^+$ T cells (lower row) are indicated in each dot plot. (B) Alignment of DNA and amino acid (aa) sequence of *VP1* C termini of all known GCN-type JCV and prototype JCV Mad-1. The CSF of our patient contains GCN1b, GCN6, and GCN7 subtypes. GCN = granule cell neuronopathy; JCV = JC virus; MS = multiple sclerosis; NHL = non-Hodgkin lymphoma; NTZ = natalizumab. Modified from Dang and Koralnik,[2] © 2013 American Neurological Association, with permission.

and 2,511. At the protein level, JCV_{GCN1b} has identical amino acid (aa) sequence as JCV_{GCN1}, JCV_{GCN6} has 1 aa deletion at position 344, and JCV_{GCN7} has a truncated new end at aa 341. Notably, JCV DNA, tested 3 months after PLEX, was not detected in the plasma, peripheral blood mononuclear cells, or urine of this patient. The sequence of JCV hypervariable regulatory region (RR) amplified from the CSF of our

Figure 3 Resolution of immune reconstitution inflammatory syndrome and spread of white matter lesions on MRI 11 months after initial presentation



(A) T1 precontrast image demonstrates significant atrophy of the cerebellar vermis. (B) FLAIR image showing worsening of the diffuse white matter hyperintensities in the brainstem (arrow). (C) T1 postcontrast image shows resolution of the cerebellar enhancement noted previously. FLAIR = fluid-attenuated inversion recovery.

patient was identical to that obtained from the initial case of JCV GCN, showing an archetype-like RR.[7]

The patient's neurologic function stabilized concomitant with negative repeat JCV CSF PCR 4 months after PLEX. MRI and ^{1}H-MRS obtained 8 months after onset of symptoms showed near resolution of cerebellar enhancement and a lipid 1/creatine ratio <1.0 in cerebellum consistent with subsiding IRIS.[8]

Eleven months after initial presentation, ataxia, nystagmus, and dysarthria persisted. MRI at that time showed cerebellar atrophy, especially in the vermis (figure 3A), along with progression of fluid-attenuated inversion recovery hyperintensities in the pons and cerebellar peduncles (figure 3B). There was no further enhancement on postcontrast images (figure 3C). Spasticity from his cervical MS lesions and the cerebellar ataxia restrict him to a wheelchair for most of the time. Maraviroc was discontinued, and his MS has remained stable on glatiramer acetate 15 months after JCV GCN onset.

DISCUSSION This is the second case of JCV GCN in a natalizumab-treated patient with MS. Both patients received more than 50 doses of natalizumab monotherapy and had progressive cerebellar symptoms without white matter changes suggestive of PML on initial MRI.[5] They were both treated with PLEX and survived with significant morbidity.

However, our patient differs from the one reported by Schippling et al.[5] in various aspects (table). Our patient had an MRI 1 month after his initial presentation, which did not identify any cerebellar atrophy, whereas it was already evident in the other case on the first MRI 4 months after symptom onset. Our patient also developed extensive white matter lesions in the pons and cerebellar peduncles 9 months after initial presentation, despite a negative JCV PCR in CSF. The follow-up in their patient lasted 5 months, during which no white matter lesions were observed.

It is therefore possible that JCV initially infected granule cell neurons, causing cerebellar atrophy, and then spread in the cerebellar and pontine white matter. Nevertheless, the stabilization of the lesions and extended survival of our patient are remarkable, commensurate with a strong cellular immune response to JCV, consistent with our observations in PML.[6] The stronger CD4 response, measured after IRIS onset, supports the role of $CD4^{+}$ T cells in IRIS in natalizumab-treated patients with MS, as it has been shown in the setting of PML.[9]

Notably, our patient developed contrast enhancement in the cerebellum on MRI in association with IRIS, contrary to the case of Schippling et al., where IRIS was diagnosed only by identification of T-cell infiltrates on cerebellar biopsy. In both cases, IRIS was triggered by the return of lymphocytes in a cerebellum heavily infected by JCV, causing local inflammation. Whether extension of lesions and clinical worsening were caused by a prolonged IRIS requiring additional corticosteroids or the natural progression of JCV GCN where corticosteroids would be

Table Comparison of 2 patients with natalizumab-associated JCV GCN

JCV GCN	Present case	Schippling et al.[5]
Length of natalizumab monotherapy	63 mo	52 mo
Cerebellar atrophy on first MRI after symptoms onset	Absent at 1 mo	Present at 4 mo
Contrast enhancement on MRI during IRIS	+	–
Cerebellar infiltrate of T lymphocytes during IRIS	NA	+
Strong JCV-specific T-cell response in blood and extended survival	+	NA
^{1}H-MRS suggesting subsiding IRIS	+	NA
Identification of GCN-type JCV mutations in CSF	+	NA
Extension of lesions in cerebellar and pontine white matter	+	–

Abbreviations: GCN = granule cell neuronopathy; ^{1}H-MRS = proton magnetic resonance spectroscopy; IRIS = immune reconstitution inflammatory syndrome; JCV = JC virus; NA = not applicable.

contraindicated created a difficult clinical conundrum in our case. We therefore used the algorithm recently delineated in PML, showing low lipid 1/creatine ratio on ^{1}H-MRS and subsiding contrast enhancement in the lesions. In PML, this constellation corresponds to a probability of IRIS of only 13%.[8] Indeed, high lipid peaks are thought to be a marker for T-cell infiltration and have been reported in various inflammatory conditions such as MS, acute disseminated encephalomyelitis, and PML-IRIS.[10,11] Whether this algorithm can be validated in JCV GCN as well will require further study.

Maraviroc is an antiretroviral medication that blocks binding of HIV to the CCR5 receptor. It is thought to have immunomodulating properties by blocking the binding of natural CCR5 ligands and has been used in HIV$^+$ and HIV$^-$ patients with PML-IRIS.[12,13] Our experience however, does not suggest that this medication is indicated in HIV$^-$ PML-IRIS cases.

Finally, we found for the first time that JCV GCN triggered by natalizumab in the context of MS is also associated with mutations in the *VP1* gene coding for the major capsid protein. We identified 2 novel mutations that help substantiate the association between GCN-type JCV and the clinical syndrome of JCV GCN. The exact mechanism of how these mutations originate and result in tropism to the granule cells is unclear. In vitro modeling revealed that the initial GCN1 mutant, also present in our patient, was replication competent and stable, but had a disadvantage for growth in glial cells compared with undeleted JCV.[3] The fact that JCV RR in our case also had an RR identical to the GCN1 isolate characterized 10 years ago is also significant, because the RR found in PML is usually hypervariable. Moreover, JCV could not be detected in the blood, peripheral blood mononuclear cells, or urine of this patient, suggesting that the JCV mutation most likely originated in the CNS.

The description in close succession of 2 cases of natalizumab-associated JCV GCN highlights the importance of recognizing this syndrome in MS and, potentially, in patients with Crohn disease.

Initial MRI, if done within the first month of symptoms, may not identify any cerebellar atrophy and only a high degree of suspicion can lead to CSF testing. However, the sensitivity of CSF JCV PCR for diagnosis of JCV GCN is unknown, and definitive proof may require cerebellar biopsy. Identification of GCN-type mutations is a key feature of differentiation with PML. In the absence of a definite treatment against JCV, early withdrawal of natalizumab followed by PLEX constitutes the only hope for the immune system to contain JCV GCN and avoid irreversible cerebellar damage. IRIS should be expected as a complication of this treatment, and timing of corticosteroid administration may be guided by ^{1}H-MRS.

AUTHOR CONTRIBUTIONS

Dr. Agnihotri was involved in indirect patient care, acquired data, analyzed and interpreted the data, and drafted and revised the manuscript, including medical writing. Dr. Dang analyzed the virologic sequence, interpreted the data, and revised the manuscript, including medical writing. Dr. Carter was involved in direct patient care, analyzed and interpreted the data, and revised the manuscript, including medical writing. Dr. Fife was involved in direct patient care, interpreted data, and revised the manuscript, including medical writing. Ms. Bord was involved in testing the immunologic response, and analyzed and interpreted the data. Ms. Batson was involved in collecting the data and interpreted the data. Dr. Koralnik was involved in conceptualizing the study, indirect patient care, analyses and interpretation of data, and drafting and revising the manuscript.

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DISCLOSURE

S. Agnihotri and X. Dang report no disclosures relevant to the manuscript. J. Carter reports that he is a member of a Data Safety Monitoring Committee for an MS clinical trial sponsored by EMD-Serono and PPD, Inc. He has done consulting work for Novartis, Inc. and for Med-IQ, Inc. through an unrestricted educational grant from Teva Pharmaceuticals. He has received research support to Mayo Clinic for MS clinic trials from Actelion Ltd., Genzyme Inc., MedImmune Inc., and Hoffmann-La Roche, Ltd. T. Fife, E. Bord, and S. Batson report no disclosures relevant to the manuscript. I. Koralnik is funded by NIH grants R01-NS047029 and NS074995, and by a research grant from Biogen Idec and the National MS Society; served on scientific advisory boards for Hoffmann-La Roche, GlaxoSmithKline, Genentech, and Merck Serono; received consulting fees from Bristol-Myers Squibb, Ono Pharmaceuticals, Merck Serono, Hoffmann-La Roche, GlaxoSmithKline, Perseid Therapeutics, Vertex Pharmaceutical, and Johnson & Johnson; is an editorial board member for the *Journal of NeuroVirology*; and receives royalties from UpToDate for topics on the management of HIV and CNS mass lesions and on PML. Go to Neurology.org for full disclosures.

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