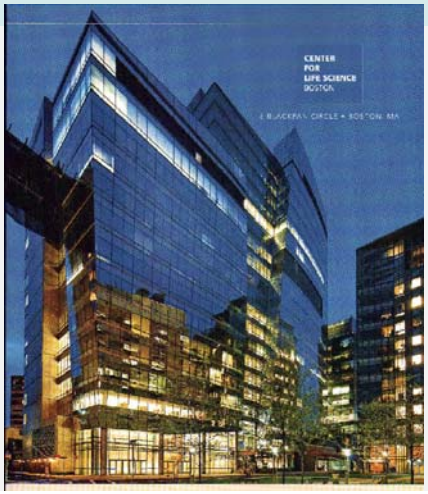
Viral subtypes and development of the disease: what is the evidence



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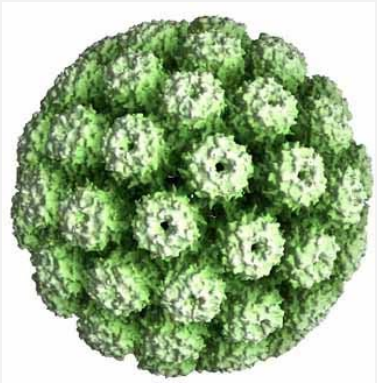
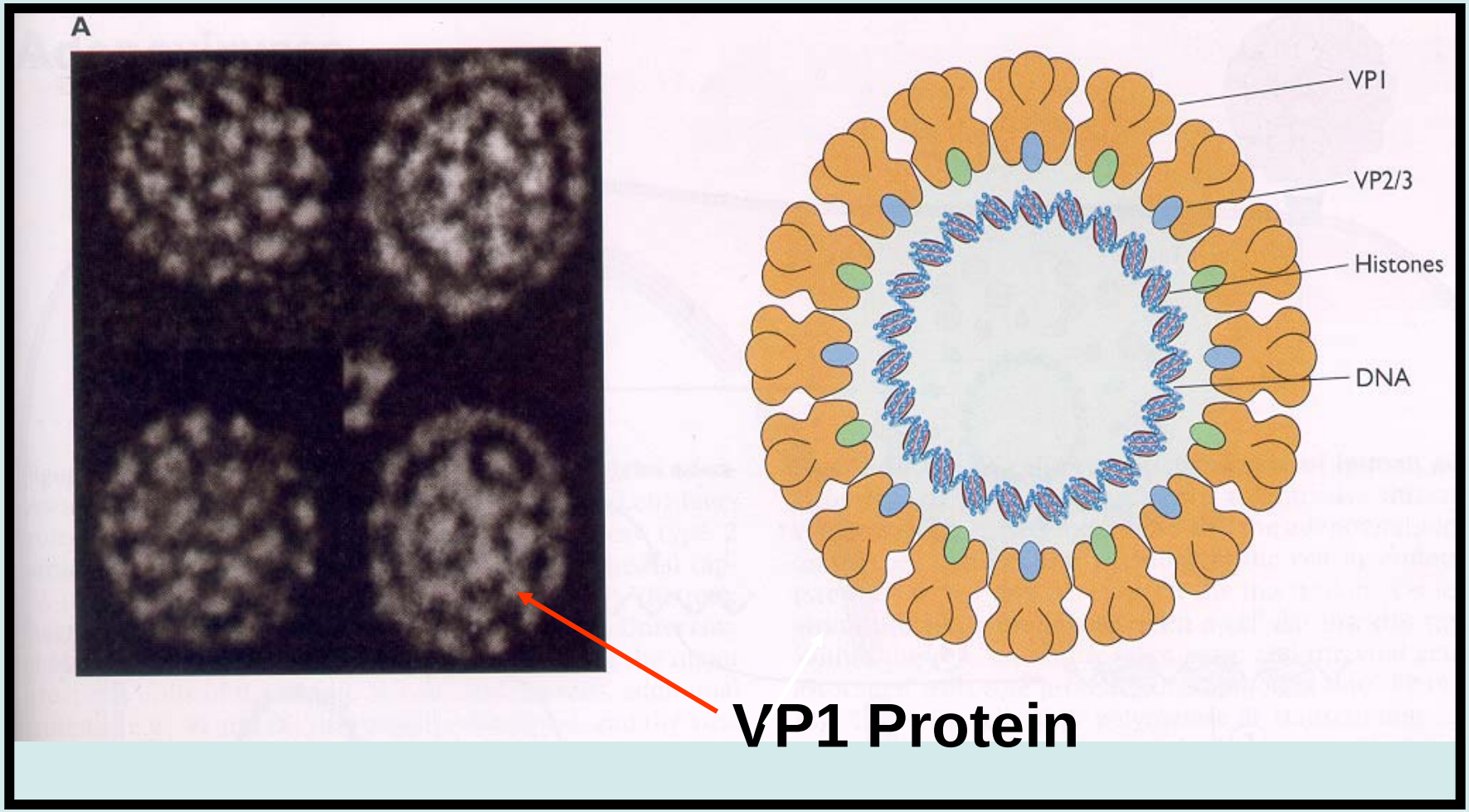
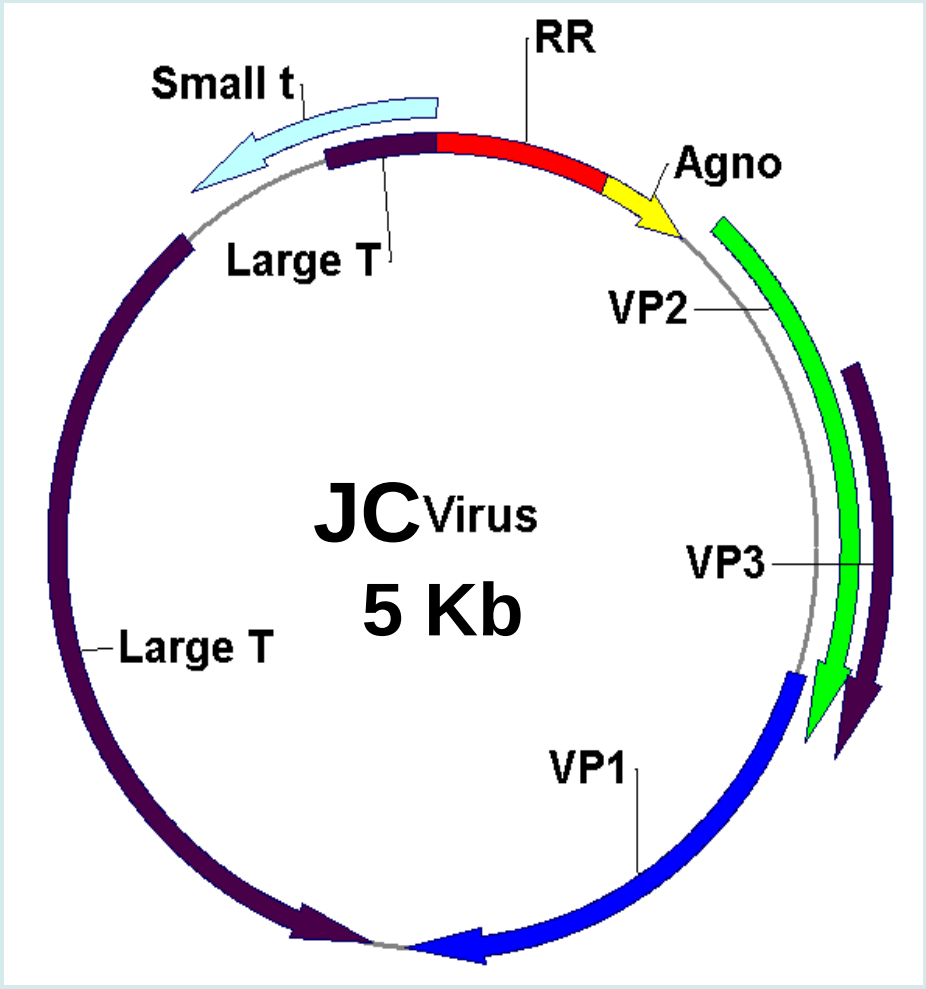


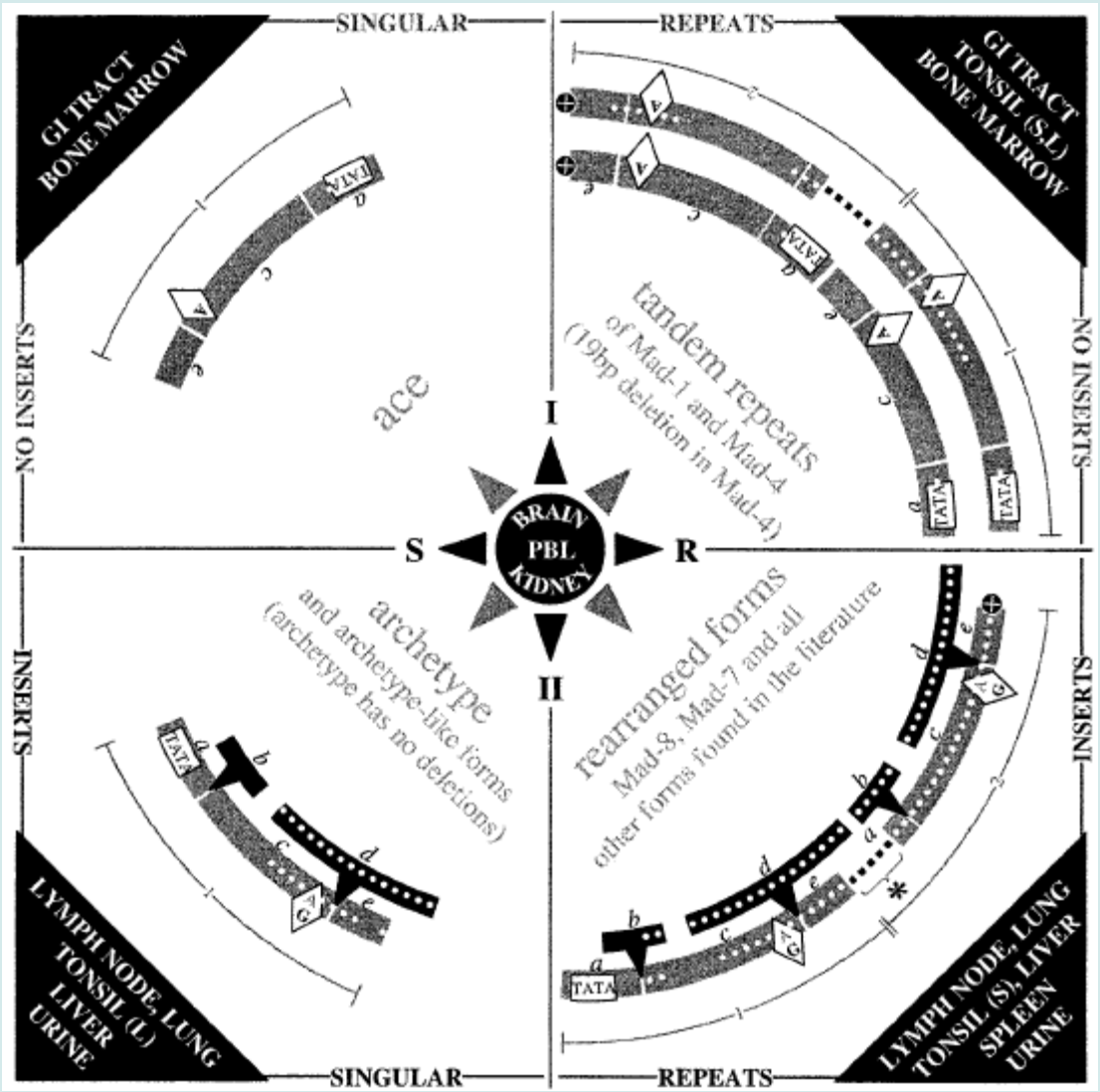
Polyomaviruses have a capsid formed by 72 pentamers of the VP1 protein



Polyomaviruses have a circular double-stranded DNA



The non coding regulatory region is hypervariable and contains determinants of neurotropism

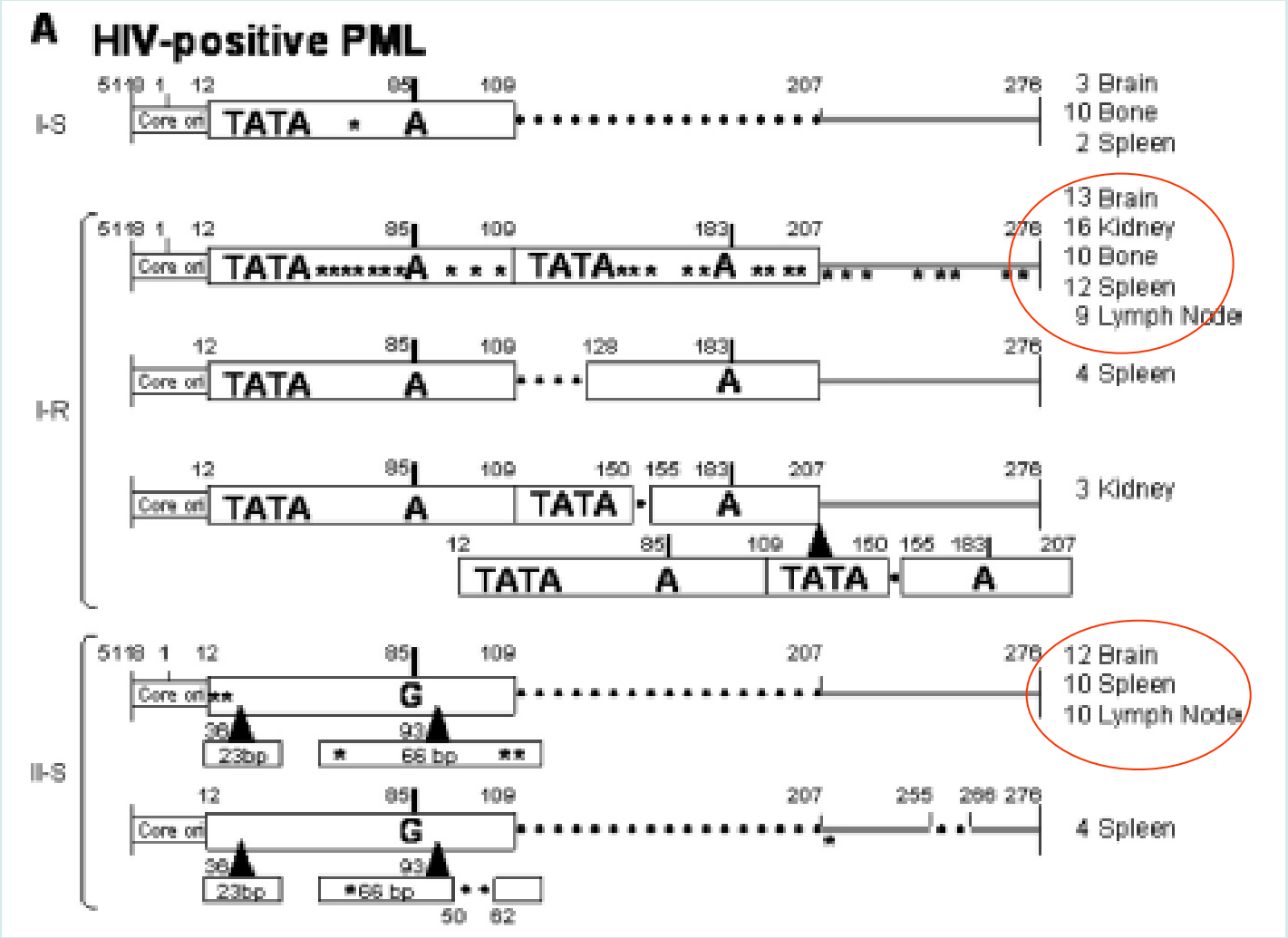


Jensen JNV 01

Archetype RR: kidney and urine of everyone

Not always …

"Rearranged" RR: CSF and brain of PML patients



Tan J Virol 10

JCV coding region is very conserved: used to follow migrations of ancient populations

- 1 serotype
- 3 genotypes (and other subtypes) based on point mutations of VP1 genes:
  - 1-4% nt mutations between different types and few aa changes
  - Type 1: Europe
  - Type 2: Asia and native Americans
  - Type 3: Africa
- Co-evolution with host: 4×10^{-7} synonymous substitutions per site/y (Hatwell J Gen Virol 00)
  - No polymorphism expected in a single person unless infected by >1 strain

Association of JCV coding region and PML

- Genotype 2B more frequent in PML lesions than urine of healthy people (Agostini JID 97)
- Comparison of isolates from PML CNS from urine isolates from healthy controls deposited in GenBank (Sunyaev PLOS Gen 09).
  - No difference in genotype
  - Half of PML isolates have at least 1 aa mutation in VP1: 55F, 60 M/E/N, 265 D/T, 267 F/L or 269 F/Y/C
  - Modeling of JCV VP1 tetrasacharide complex suggest that an implicated with binding receptor
  - VLP containing F55 and F269 variants failed to hemagglutinate Type O RBC
- Conclusion: lesser binding of JCV in the periphery may promote entry in the brain

- Caveats:
  - Most published sequences obtained from analysis of a single clone or direct sequencing of PCR products
  - Comparison of CNS PML isolates with urine isolates of HC, not with urine isolates from same PML pts
  - Structural modeling based initially on Murine PyV, then adapted to JC virus when crystal structure became available

Association of JCV coding region mutations and PML (2)

- Paired urine, plasma and CSF sample from PML patients (Gorelik JID 11)
- VP1 gene analyzed by PCR followed by direct sequencing or cloning and sequencing
  - 17 cases: but only 7 with JCV cloned from both urine and CSF
  - 2/7: 122R, 3/7: 269F, 1/7: 55F, 1/7: no change
  - Most CSF samples identical to plasma samples
  - VLP with SOME of these mutations, but NOT ALL
    - have decreased binding to gangliosides
    - fail to hemagglutinate type O RBC
    - Have decreased binding to kidney cells and lymphocytes, but not astroglial cells (not the case of 269F)
    - Binding not affected by neuraminidase treatment of astroglial cells : sialic acid independent

- Conclusion:
  - Mutations leading to lack of sialic acid binding results in escape from trapping of the virus in the periphery and access to the CNS but does not affect binding to astroglial cells
- Caveats:
  - Comparison with wild type Mad-1: also a CNS isolate
  - Limited # paired urine/CSF samples
  - Many mutations with different properties
  - PBMC not tested, only plasma
  - statistical significance ? (multiple comparisons)
  - Mutations occurring before/after virus entry to CNS ?

Association of JCV coding region mutations and PML in natalizumab-treated patients

- Paired urine, plasma and CSF sample from 17 natalizumab-treated PML patients (Reid JID 11)
- RR, VP1 gene or full length sequence analysis by PCR followed by direct sequencing or cloning and sequencing
  - 17 cases: but only 5 with both urine and CSF
  - 1 each 55F, 61P, 66H, 267L, 269F present in CSF but not urine
  - Only 4/6 paired CSF plasma samples have same mutations
  - No VP1 mutations in 6 urine samples
- Full length genome analysis in 6pts, 2-45 clones
  - 1 genotype/patient, but no association genotype/PML
  - CSF JCV “more heterogeneous than urine JCV”
  - RR rearrangements independent from VP1 mutations
  - VP1 mutations always concomitant with RR rearrangements

- Conclusion:
  - JCV in natalizumab-PML similar to other PML cases
  - RR rearrangements more common and appear to precede VP1 mutations
  - No specific association RR rearrangement / VP1 mutation
  - RR rearrangements similar in plama/CSF
  - Common asymptomatic infection with archetype in kidney with rare occurrence of change in RR and VP1 leading to CNS infection
- Caveats:
  - Limited # paired urine/CSF samples, multiple VP1 mutations
  - All samples collected at time of PML diagnosis, unclear whether VP1 mutations occur prior to clinical manifestations of PML
  - Route of primary infection not determined
  - Unclear if primary infection with archetype or rearranged RR +/- VP1 mutations

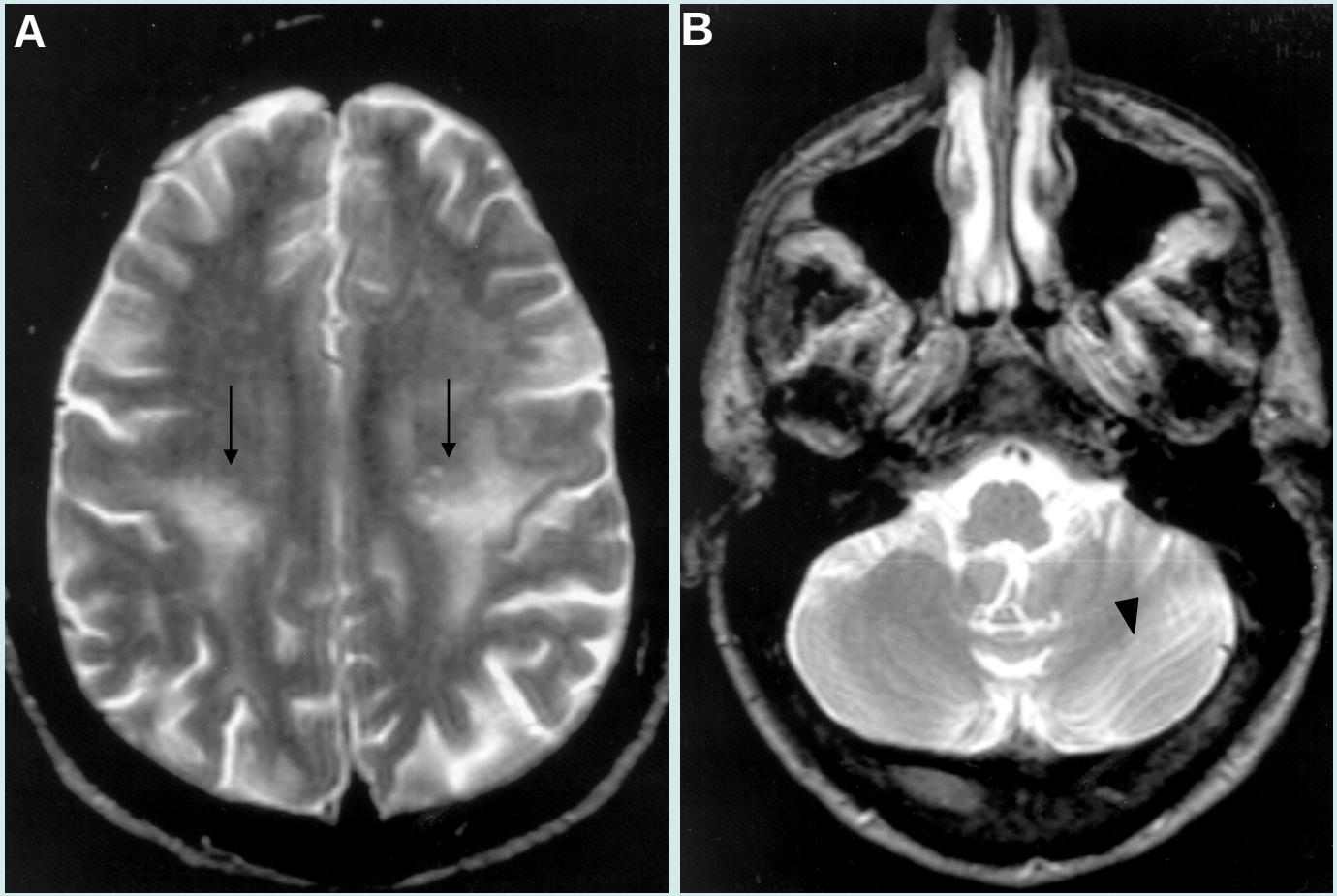
Association of JCV coding region mutations : Solutions

- Cloning and sequencing of multiple JCV clones/patient
- Full length PCR amplification and sequencing of multiple clones of JCV in a single pt at multiple time points needed to evaluate genetic evolution and intra-patient diversity
- Analysis of the data already obtained from multiple clones from MS/PML patients
- Determine JCV VP1 sequence:
  - larger number of urine/plasma/PBMC/CSF in PML patients over time
  - urine/plasma/PBMC in immunosuppressed patients without PML over time
  - urine of natalizumab-treated MS patients over time
- Longitudinal studies with deep sequencing of JCV in different compartments in natalizumab-treated patients

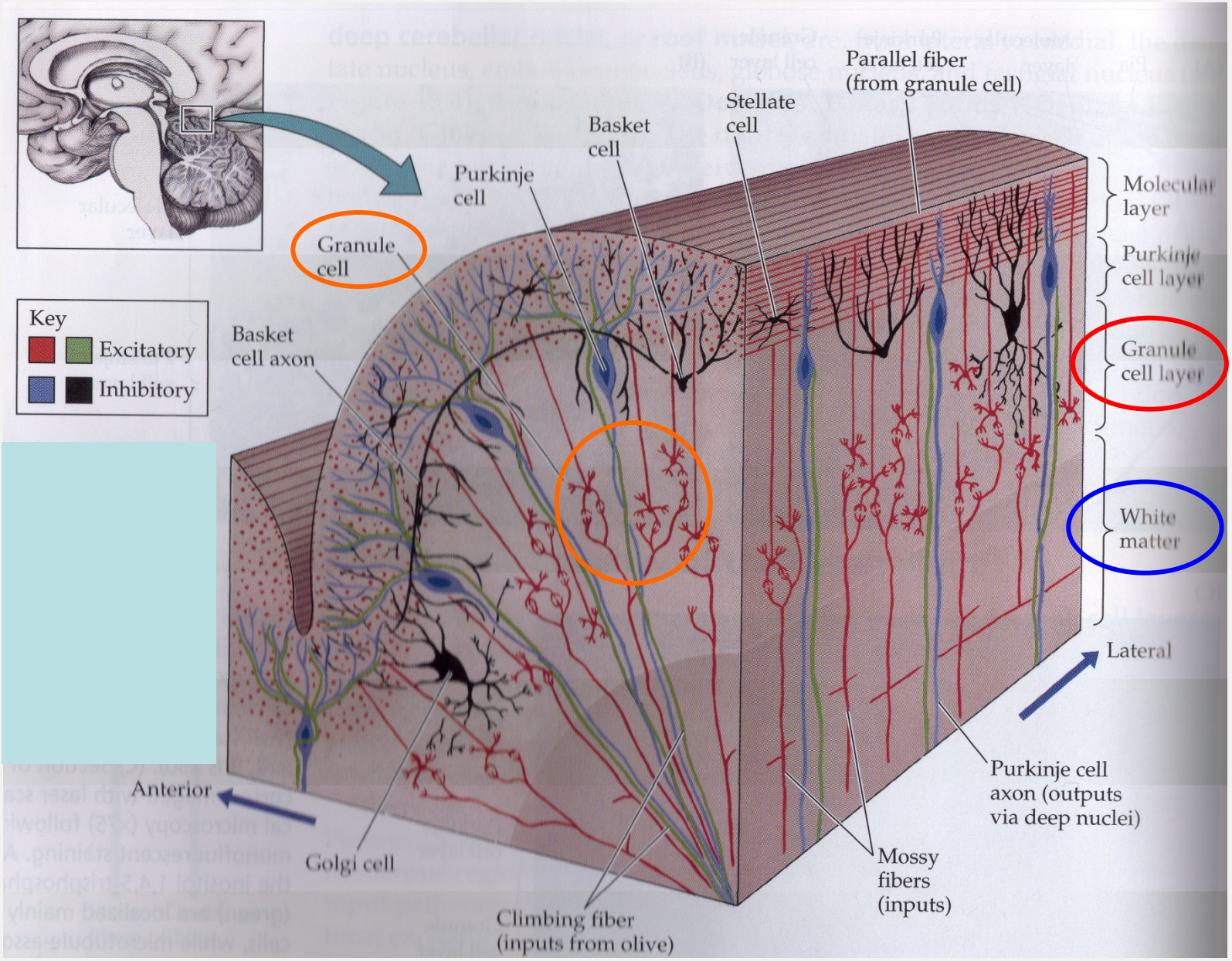
JCV can also infect neurons: 2 novel syndromes caused by JCV variants

- Areas of focal cell loss in the internal granule cell layer (IGCL) of the cerebellum in 5% of patients with PML before the AIDS era (Richardson Prog Clin Biol Res,1983**)**
- Idiopathic cerebellar atrophy in AIDS with loss of cells of the IGCL **(Tagliati Neurology, 1998)**

HIV+ patient with PML and cerebellar atrophy



Granule cell neurons (GCN)

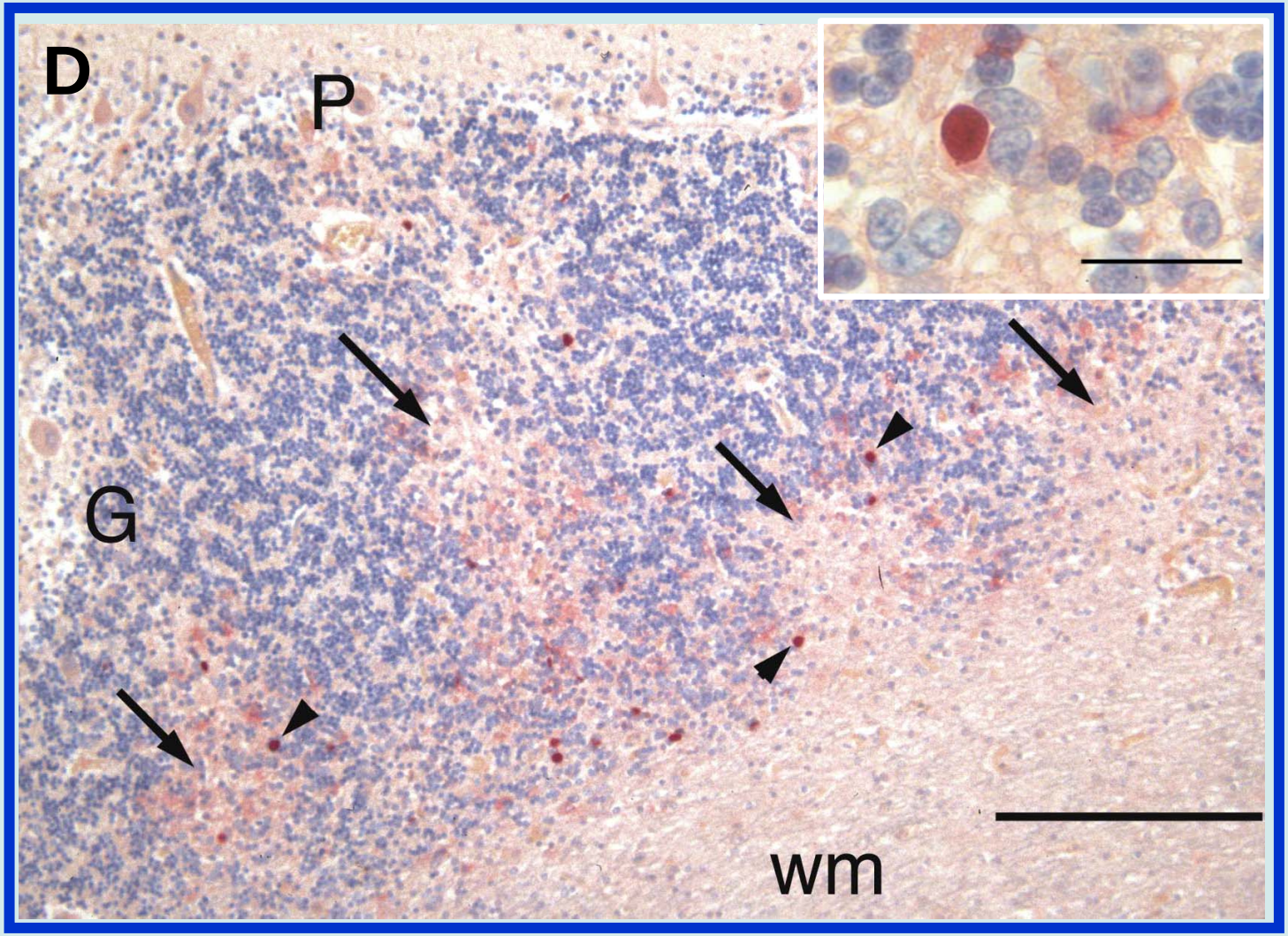


Granule cell neurons

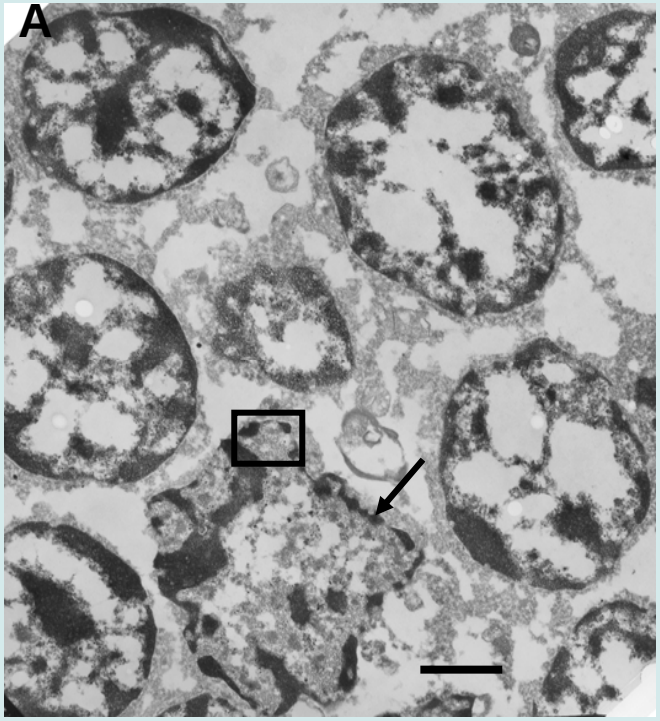
PML lesions

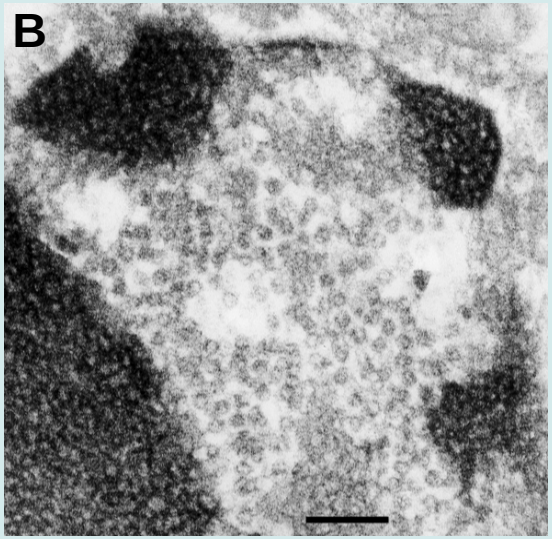
Granule Cell Neurons are infected by JCV

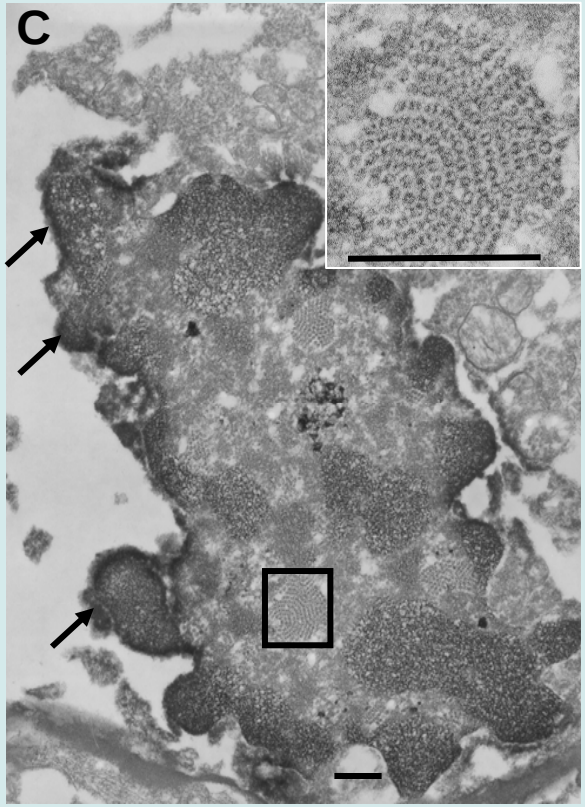
Du Pasquier et al. Neurol 2003



Mature JC virions are present in granule cell neurons



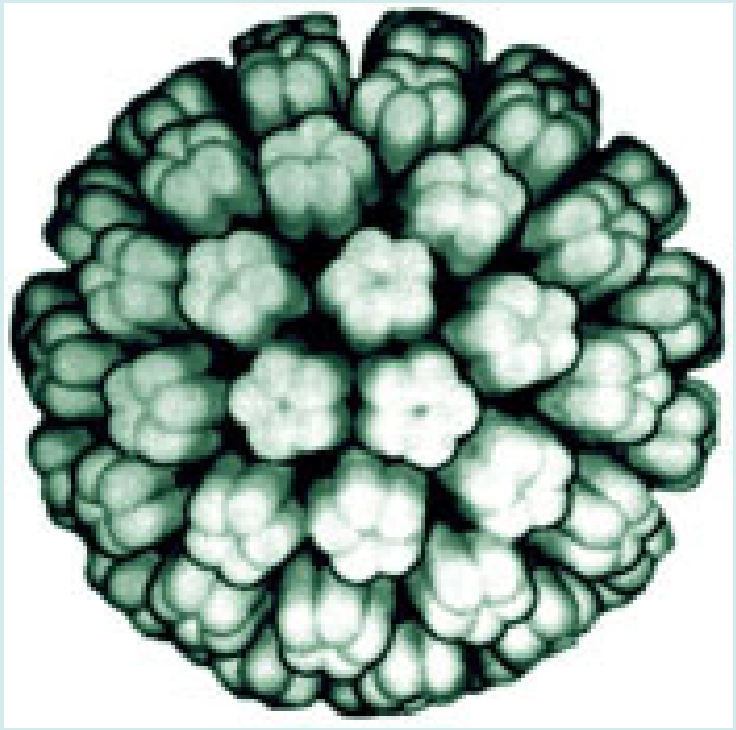


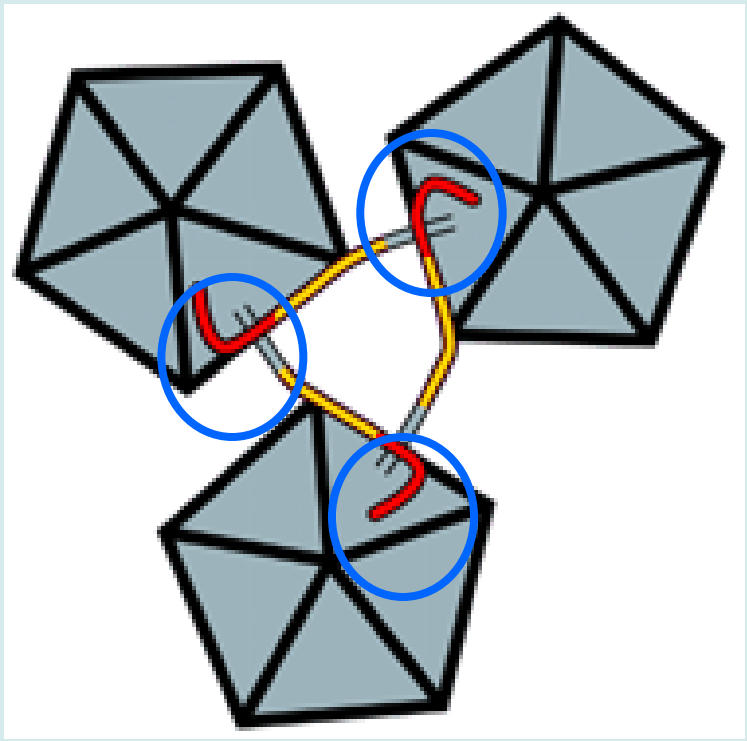


Molecular basis of GCN tropism

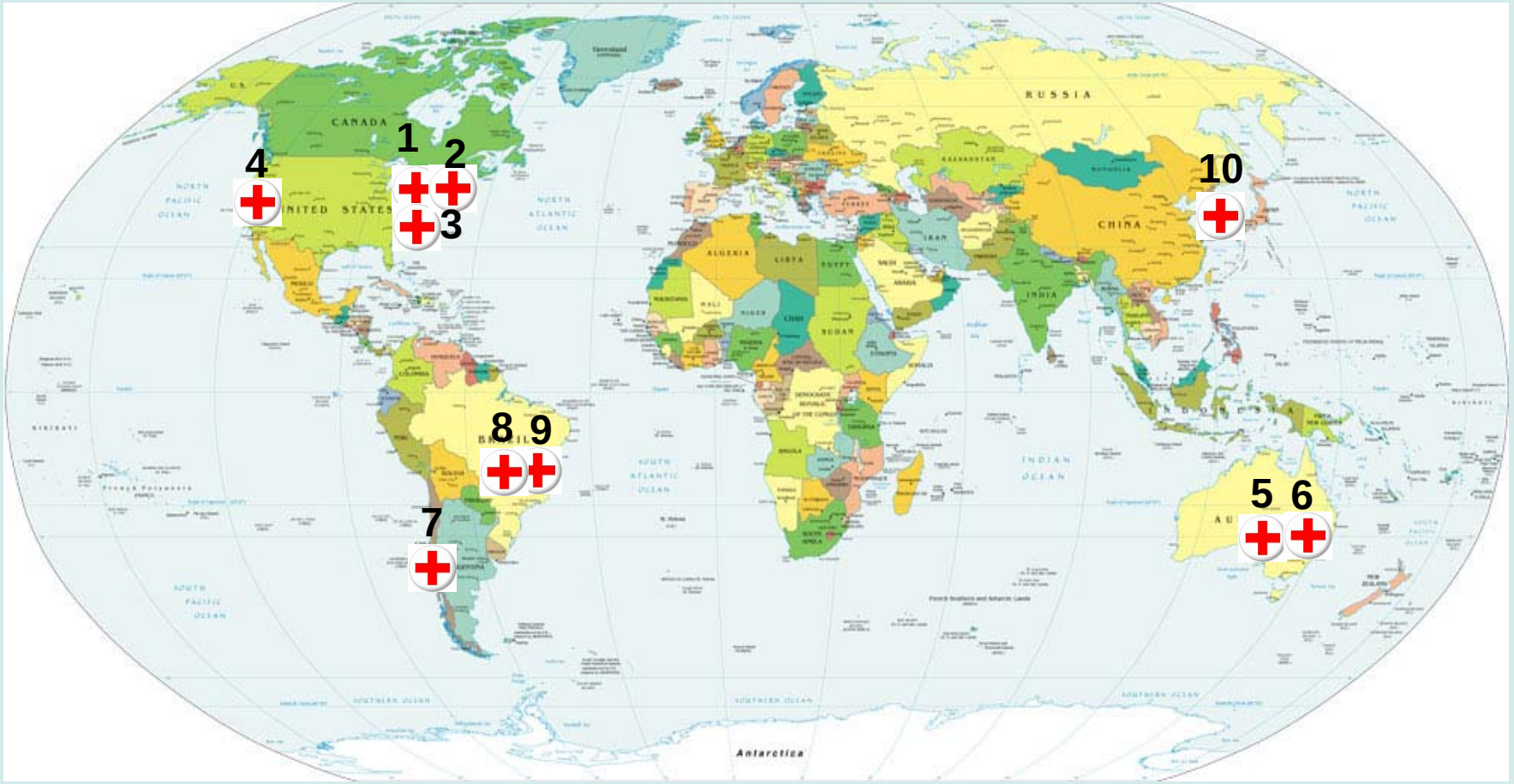
Dang et al. J Gen Virol 2006

- 10 bp out of frame deletion in JCV VP1 protein, resulting in change of 13 aa at C terminus

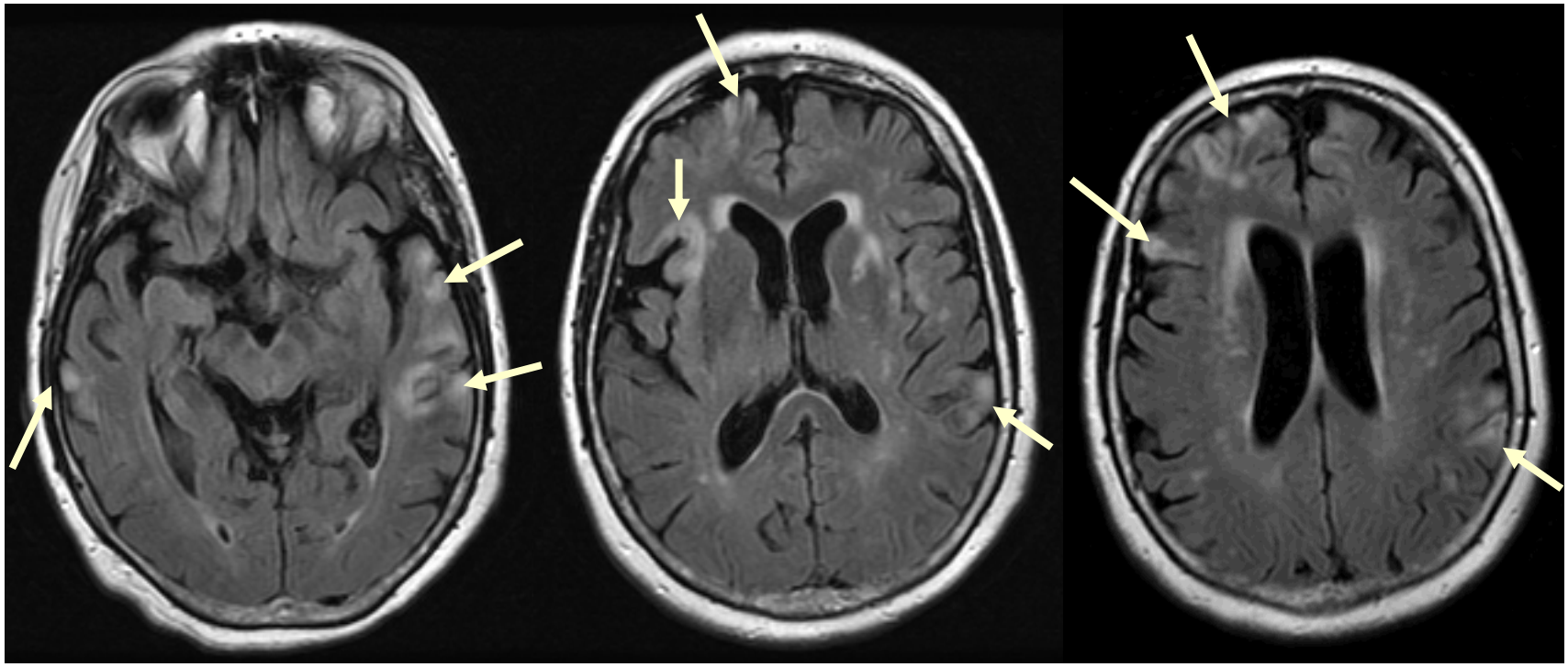




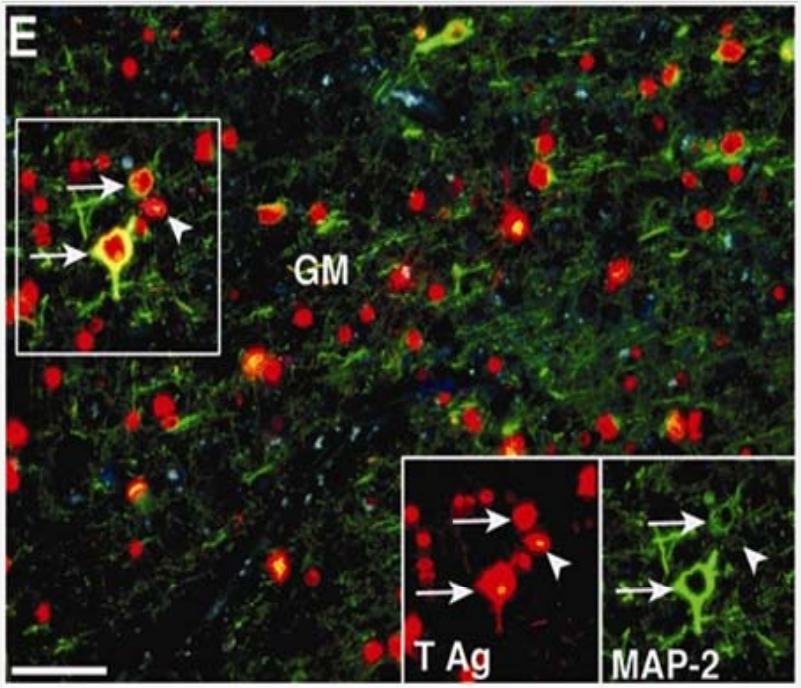
10 case-reports of JCV Granule Cell Neuronopathy (JCV GCN)

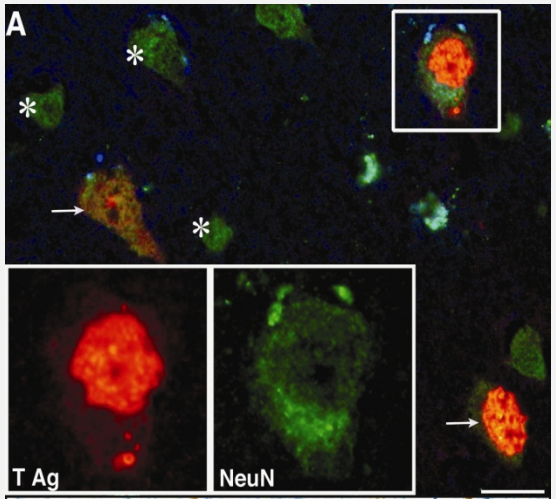


74 yo HIV negative lady with gray matter lesions and encephalopathy

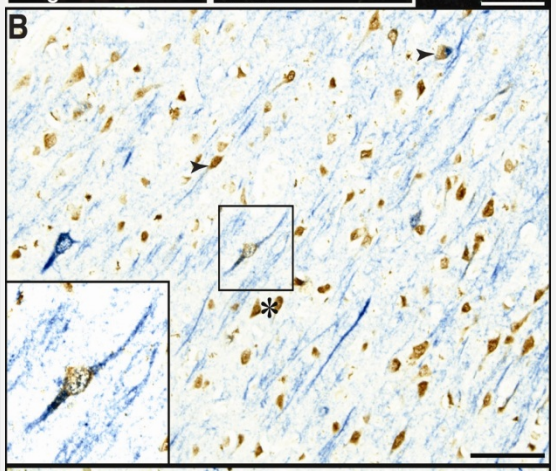


Colocalization
JCV T Ag
MAP-2



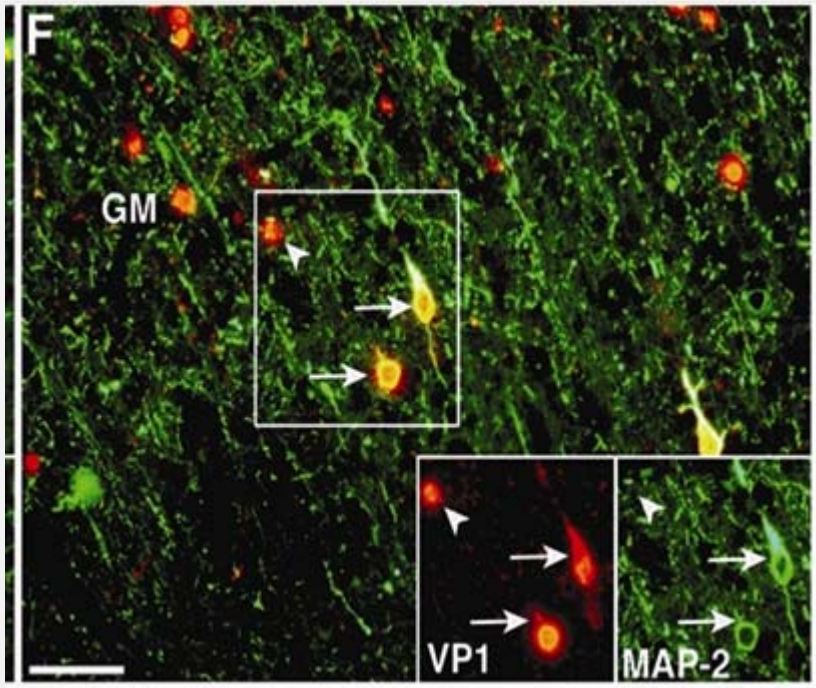


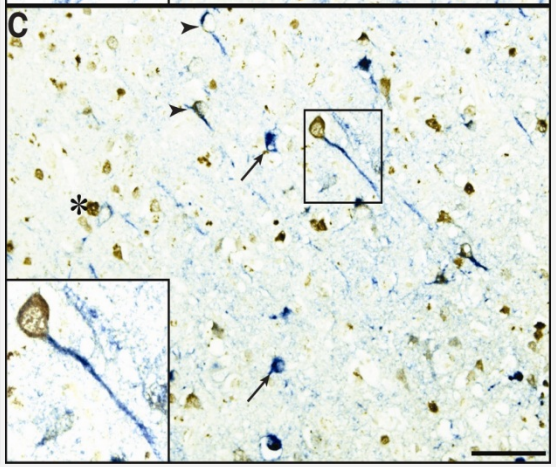
Colocalization
JCV T Ag
NeuN



Axonal
JCV T Ag
NeuN

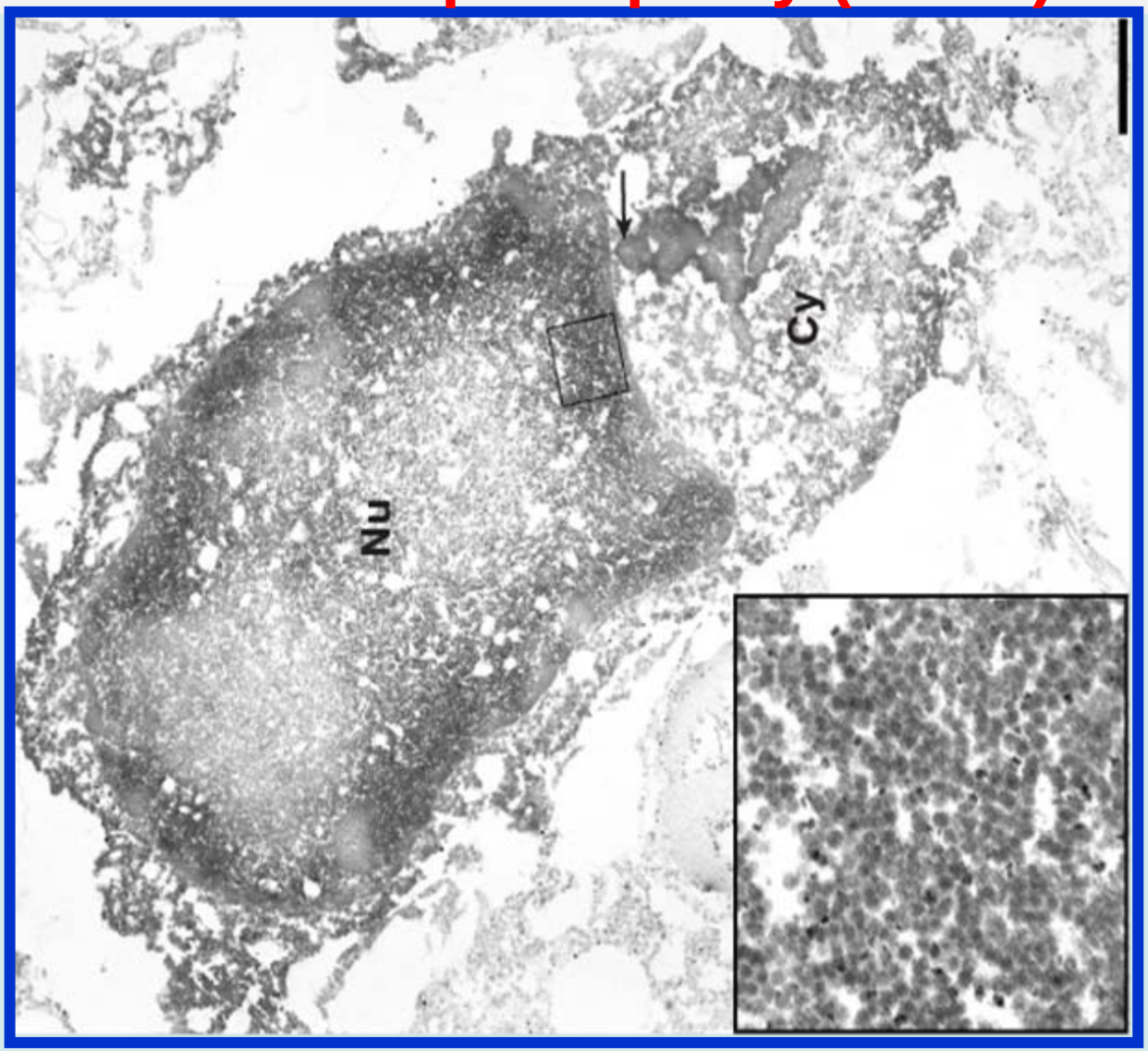
Colocalization
JCV VP1
MAP-2





Axonal
JCV VP1
NeuN

Mature JC virions are present in nuclei of cortical pyramidal neurons:
JCV Encephalopathy (JCVE)



Wuthrich
Ann Neurol 09

Clinical presentation	Classic PML		JCV GCN	JCVE
Onset	**Subacute**		**Chronic**	**Subacute**
MRI	**Subcortical lesions**		**Cerebellar atrophy**	**Cortical lesions**
Neurologic symptoms	**Based on location**		**Cerebellar syndrome**	**Encephalopathy**
Histology	**Demyelination**		**Lytic infection of granule cell neurons**	**Lytic infection of pyramidal neurons**
JCV sequence	**Single aa mutations in VP1**		**Alterations in VP1 C terminus**	**Novel mutation in Agnogene**

Tan CS Lancet Neurol 2010

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  - Laura Ellis
  - Yiping Chen
  - Evelyn Bord
  - Elizabeth Norton
  - Angela Marzocchetti
  - Thomas Broge

PML patients and their families

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  - Susan Morgello
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NINDS R01 041198 and 047029, K24 060950, R21 051124, Harvard CFAR