Host Cell Specific Interactions with JCPyV Noncoding Control Region in Progressive Multifocal Leukoencephalopy

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Introduction

JC Polyomavirus (JCPyV) infects 40-60% of the population worldwide. In healthy individuals, it is asymptomatic and takes residence in the kidney with occasional shedding in the urine. In immunocompromised patients it can travel to the central nervous system (CNS) causing a debilitating and often lethal infection known as Progressive Multifocal Leukoencephalopathy (PML). There is no available treatment for JCPyV and patients who survive the infection are left with severe neurological deficits[1].

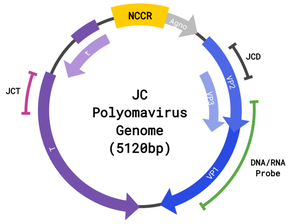


Figure 1. Simplified diagram of JCPyV genome Early genes are given in purple and late genes in blue. Regions of PCR amplification (JCD and JCT) and probe detection are given.

Progression to neurologic disease is thought to be associated with rearrangement of the noncoding control region (NCCR). Cases of PML and Granule Cell Neuronopathy (GCN) generally contain several insertions and deletions in this region while virus in the kidney known as archetype does not contain these changes[2,3]. Here we attempt to identify how NCCR rearrangements affect viral replication and protein expression

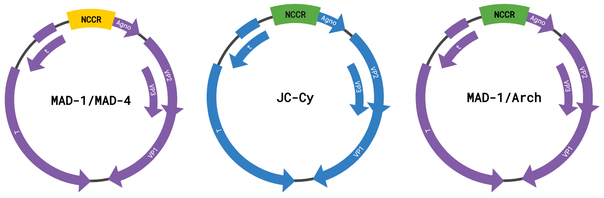
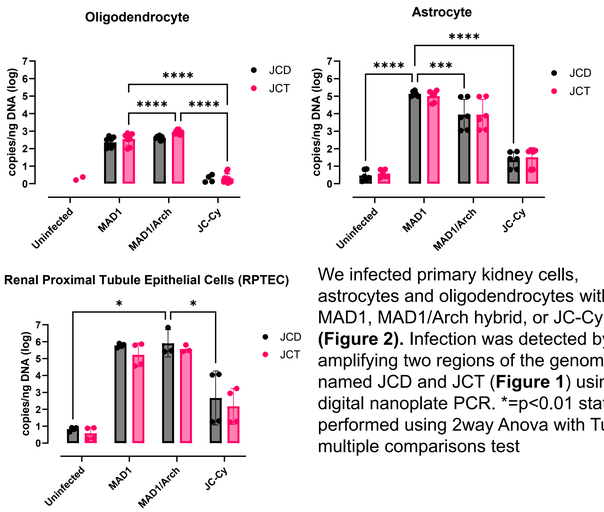


Figure 2. JCPyV variants. We used wildtype strains of archetype (JC-Cy) and neurotropic JCPyV (MAD-1) with nonrearranged and rearranged NCCR, respectively, as well as a hybrid neurotropic virus with a nonrearranged NCCR (MAD-1/Arch).

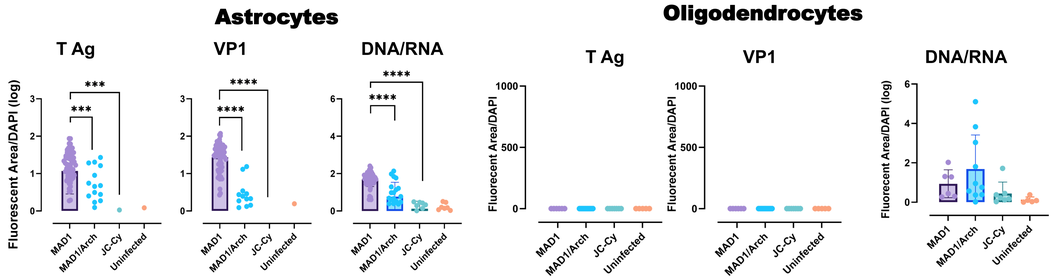
Rearrangement improves JCPyV infection of Astrocytes



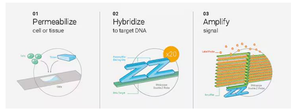
We infected primary kidney cells, astrocytes and oligodendrocytes with MAD1, MAD1/Arch hybrid, or JC-Cy **(Figure 2).** Infection was detected by amplifying two regions of the genome named JCD and JCT (**Figure 1**) using digital nanoplate PCR. *=p<0.01 statistics performed using 2way Anova with Tukey's multiple comparisons test

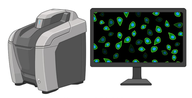
Results

Viral protein expression is increased with NCCR rearrangement

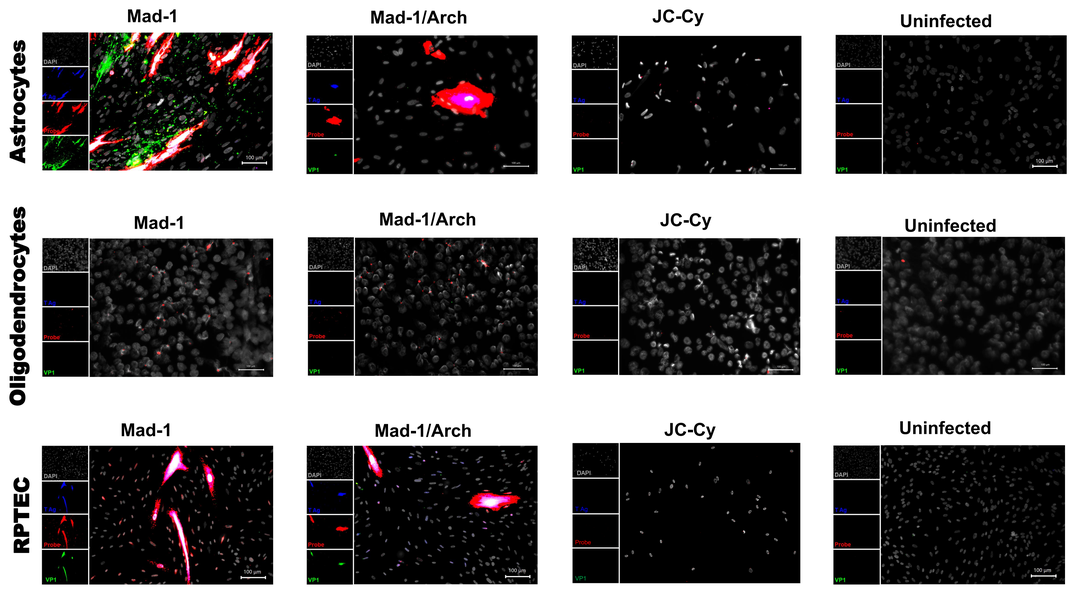


We infected primary renal proximal tubular epithelial cells (RPTECs), oligodendrocytes, and astrocytes with MAD-1, MAD-1/Arch, or JC-Cy (**Figure 3**). Cells were stained for early protein (T Ag) and late protein (VP1) as well as a DNA/RNA probes. Fluorescent area was calculated and normalized to DAPI. Significance calculated by one-way ANOVA and multiple comparisons test * p<0.05 *** p<0.0004 **** p<0.0001




Keyence BZ-X800 system

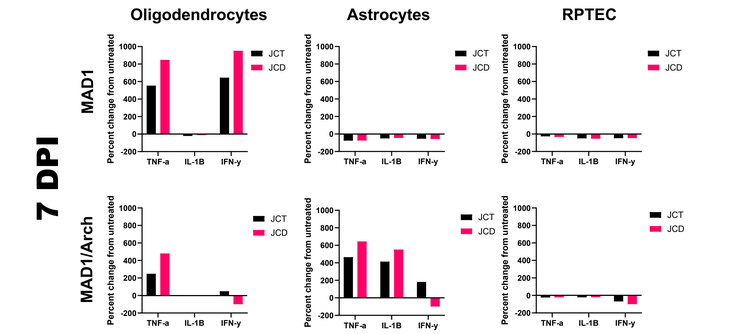
Immunofluorescence shows phenotypic differences between NCCR rearrangement and host cell types



Immunofluorescence Images were taken at 20x on a Keyence BZ-X800 microscope and imaging setting were kept consistent across specimens.

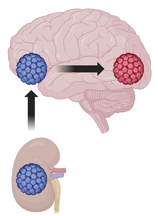
Results Continued

NCCR rearranges to minimize regulation by inflammatory cytokines



CNS and kidney cells were treated or pretreated with cytokines during JCPyV infection for 7 days, 10 days (oligodendrocytes), or 12 days (RPTEC and Astrocytes). Following infection DNA was extracted and dPCR was used to amplify two regions of the genome (Figure 1). The average percent change from untreated was calculated.

Conclusions and hypotheses



- Archetype JCPyV enters the CNS prior to disease
- Astrocytes are the main CNS cell infected by JCPyV
  - Astrocyte infection may be responsible for disease
- NCCR rearranges to better infect astrocytes and evade the immune system
- NCCR rearrangements are host cell specific

Future Directions

- Identify changes in host cell response to NCCR rearrangements by scRNA-seq
- Pull down NCCR to identify which proteins are binding during infection
- Replicate results of cytokine assay looking at infectious virion and viral protein production
- Identify how virus infection relates to cell death and demyelination in PML

References
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Graphics from BioRender.com and ACDbio.com