I am pleased to provide you complimentary one-time access to my article as a PDF file for your own personal use. Any further/multiple distribution, publication or commercial usage of this copyrighted material would require submission of a permission request to the publisher.

Timothy M. Miller, MD, PhD
Professor of Neurology
Washington University School of Medicine
Tau positron emission tomography imaging in C9orf72 repeat expansion carriers


Keywords: amyotrophic lateral sclerosis, AV-1451, C9orf72, positron emission tomography, tau

Received 17 October 2018
Accepted 21 January 2019

European Journal of Neurology 2019, 0: 1–5
doi:10.1111/ene.13940

Background and purpose: AV-1451 (18F-AV-1451, flortaucipir) positron emission tomography was performed in C9orf72 expansion carriers to assess tau accumulation and disease manifestation.

Methods: Nine clinically characterized C9orf72 expansion carriers and 18 age- and gender-matched cognitively normal individuals were psychometrically evaluated and underwent tau positron emission tomography imaging. The regional AV-1451 standard uptake value ratios from multiple brain regions were analyzed. Spearman correlation was performed to relate the AV-1451 standard uptake value ratio to clinical, psychometric and cerebrospinal fluid measures.

Results: C9orf72 expansion carriers had increased AV-1451 binding in the entorhinal cortex compared to controls. Primary age-related tauopathy was observed postmortem in one patient. AV-1451 uptake did not correlate with clinical severity, disease duration, psychometric performance or cerebrospinal fluid markers.

Conclusion: C9orf72 expansion carriers exhibited increased AV-1451 uptake in entorhinal cortex compared to cognitively normal controls, suggesting a propensity for primary age-related tauopathy. However, AV-1451 accumulation was not associated with psychometric performance in our cohort.

Introduction

The C9orf72 hexanucleotide repeat expansion is the most common genetic cause of amyotrophic lateral sclerosis (ALS) and fronto-temporal dementia (FTD). Nearly 50% of patients with C9orf72-related ALS develop cognitive impairment [1].

Cognitively impaired C9orf72 expansion carriers have been described with accumulation of intracellular tau fibrils [2,3] and a pathological burden of neurofibrillary tangles consistent with Alzheimer’s disease (AD) [3]. Other studies found minimal tau pathology in C9orf72-positive patients across the ALS/FTD spectrum [2].

Positron emission topography (PET) tracers that bind to aggregated tau, including AV-1451 (18F-AV-1451, T807, flortaucipir), provide a novel non-invasive approach for investigating the spatial accrual of tau [4] and have shown promise as biomarkers for disease progression in AD [5] and other tauopathies [6]. Here, the AV-1451 uptake in C9orf72 expansion carriers was studied and the relationships between AV-1451 binding and clinical features were examined.
Materials and methods

Participants

The study was approved by the Washington University in Saint Louis (WU) Institutional Review Board and was registered at clinicaltrials.gov (NCT02414230). Participants (n = 9) were recruited from the WU neuromuscular clinic and provided written informed consent. All had an expanded C9orf72 allele by repeat-primed polymerase chain reaction through a laboratory approved according to the Clinical Laboratory Improvement Amendments. None was diagnosed with FTD. Clinical history, physical examination and a revised ALS Functional Rating Scale (ALSFRS-R) assessment were collected. The ALS Cognitive Behavioral Score was obtained from the medical record.

Eighteen cognitively normal participants (mean age 63.9 ± 5.4 years, 50% men) were recruited from the WU Knight Alzheimer’s Disease Research Center to match our experimental cohort 2:1. Controls had a clinical dementia rating score of zero and were amyloid-negative on imaging with18F-AV-45.

Neuropsychological tests administered included the Mini-Mental State Examination (MMSE), Trailmaking A (TMA), Trailmaking B (TMB), Letter-Number Sequencing, category fluency (animals) and the logical memory subtest from the Wechsler Memory Scale III. One C9orf72 expansion carrier was unable to perform neuropsychological testing due to advanced disease. Four additional participants did not complete the logical memory subtest due to speech limitations.

Cerebrospinal fluid AD biomarker analysis

Cerebrospinal fluid (CSF) Aβ42, total tau (t-tau) and phospho-tau (p-tau) were analyzed by enzyme-linked immunosorbent assay (INNOTEST®, Fujirebio-Europe; Ghent, Belgium) [7].

Brain imaging

Structural magnetic resonance imaging (MRI) scans were acquired using a Biograph mMR PET-MRI scanner. AV-1451 PET scans were acquired on a Siemens Biograph 40 PET-CT scanner for controls or a Biograph mMR PET-MRI scanner for C9orf72 expansion carriers. Attenuation correction, tracer analysis, volumetric segmentation and partial volume correction were performed as previously described [8].

Scanner specific spatial filters were applied to achieve a common resolution (8 mm³) across PET scanners [9,10]. Regional standard uptake value ratios (SUVRs) were obtained using the cerebellar gray matter as a reference region during the 80–100 min post-injection time. Additional comparisons to the brainstem and cortical regions were performed.

Neuropathological analysis

Brain and spinal cord sections were analyzed by the Anatomic and Molecular Pathology Core Laboratory at WU.

Statistical analyses

AV-1451 SUVRs were compared by independent two-tailed t tests using a Bonferroni corrected P value of 0.0042 (adjusted P value = 0.05/12) to correct for multiple comparisons. Spearman correlation was used to relate AV-1451 uptake to clinical/paraclinical measures.

Results

Clinical and neuropsychological characteristics

C9orf72 expansion carriers varied in site of onset, disease duration and ALSFRS-R scores. Two participants, #2 and #7, did not display motor impairment (Table 1). C9orf72 expansion carriers performed worse on a global measure of neuropsychological performance (Mini-Mental State Examination) and required longer times to complete TMA (control 27.39 ± 8.49; C9orf72 45.96 ± 12.56, P = 0.0003) and TMB (control 64.06 ± 29.35; C9orf72 105.5 ± 43.86, P = 0.011) tasks. TMA/TMB performance was not related to motor impairment from the ALSFRS-R handwriting subscore (TMA, Spearman r = −0.232, P = 0.529; TMB, Spearman r = −0.020, P = 0.781). Thus, C9orf72 expansion carriers had cognitive deficits in attentional and executive domains.

AV-1451 SUVRs in regions commonly affected by C9orf72-related disease were examined based on prior morphometric or pathological studies [2,3,11]. The entorhinal cortex displayed elevated AV-1451 SUVRs in C9orf72 expansion carriers (C9orf72, 1.347 ± 0.376; control, 0.982 ± 0.199; P = 0.0027; Fig. 1a, b). No other targeted region of interest displayed increased AV-1451 uptake (Fig. 1b). Since cerebellar pathology has been described in C9orf72-related disease [11], comparisons using AV-1451 SUVRs normalized to brainstem and mean cortical regions were also performed and revealed increases in entorhinal tau deposition (data not shown).

Neither ALSFRS-R (r = 0.357, P = 0.444) nor disease duration (r = 0.571, P = 0.200) correlated with entorhinal AV-1451 uptake suggesting that AV-1451 uptake is not associated with physical decline.
Trailmaking A/Trailmaking B performance has been associated with activation of frontal regions [12]. Relationships between TMA and TMB performance were examined with AV-1451 SUVRs in the entorhinal (TMA, $r = -0.071, P = 0.906$; TMB, $r = 0.571, P = 0.2$), caudal middle frontal (TMA, $r = -0.5, P = 0.267$; TMB, $r = -0.8, P = 0.45$), and superior frontal (TMA, $r = -0.7, P = 0.06$; TMB, $r = -0.5, P = 0.267$).

<table>
<thead>
<tr>
<th>Participant</th>
<th>Age</th>
<th>Gender</th>
<th>Site of onset</th>
<th>Disease duration (years)</th>
<th>ALSFRS-R (/48)</th>
<th>MMSE (/30)</th>
<th>ALS-CBS (/21)</th>
<th>Ed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>64</td>
<td>Female</td>
<td>Spinal</td>
<td>1.57</td>
<td>13</td>
<td>N/A</td>
<td>N/A</td>
<td>B</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>Male</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>30</td>
<td>M.S.</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>Male</td>
<td>Bulbar</td>
<td>3.67</td>
<td>22</td>
<td>27</td>
<td>10</td>
<td>N/A</td>
</tr>
<tr>
<td>4</td>
<td>64</td>
<td>Male</td>
<td>Spinal</td>
<td>4.19</td>
<td>21</td>
<td>23</td>
<td>16</td>
<td>M.S.</td>
</tr>
<tr>
<td>5</td>
<td>69</td>
<td>Female</td>
<td>N/A</td>
<td>2.67</td>
<td>25</td>
<td>24</td>
<td>8</td>
<td>Ph.D.</td>
</tr>
<tr>
<td>6</td>
<td>57</td>
<td>Female</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>25</td>
<td>N/A</td>
<td>GED</td>
</tr>
<tr>
<td>7</td>
<td>68</td>
<td>Female</td>
<td>Bulbar</td>
<td>2.25</td>
<td>39</td>
<td>30</td>
<td>20</td>
<td>HS</td>
</tr>
<tr>
<td>8</td>
<td>60</td>
<td>Male</td>
<td>Spinal</td>
<td>1.94</td>
<td>36</td>
<td>30</td>
<td>N/A</td>
<td>HS</td>
</tr>
</tbody>
</table>

Avg 63.0 ± 4.0 56% Male N/A 2.50 ± 1.09 28 ± 9.8 27.4 ± 3.0 14.2 ± 5.0

Control cohort demographics (n = 18)
63.5 ± 5.3 50% Male N/A N/A N/A 29.9 ± 0.4 N/A N/A

ALS-CBS, Amyotrophic Lateral Sclerosis Cognitive Behavioral Score; ALSFRS-R, revised Amyotrophic Lateral Sclerosis Functional Rating Scale; Ed, educational level denoted Bachelor's degree (B), Master's degree (M.S.), Doctorate (Ph.D.), High School (HS) or General Educational Development Test (GED). MMSE, Mini-Mental State Examination; N/A, not available.
Cerebrospinal fluid Aβ42 levels (CSF Aβ42: 973 ± 443 pg/ml) were within ranges reported for cognitively normal and amyloid-negative individuals [7]. CSF biomarker levels did not correlate with entorhinal AV-1451 uptake in C9or72 patients (Aβ, \( r = 0.543 \), \( P = 0.297 \); t-tau, \( r = 0.257 \), \( P = 0.658 \); p-tau, \( r = 0.257 \), \( P = 0.658 \)).

Postmortem analysis was performed on tissue from participant #3 (entorhinal SUVR 1.26). Staining for p-tau showed neurofibrillary tangles primarily within the entorhinal cortex. Entorhinal cortical sections did not exhibit β-amyloid deposition (Fig. 1c), consistent with primary age-related tauopathy (PART).

**Discussion**

Tau PET imaging in C9or72 expansion carriers with motor and cognitive deficits revealed an increased tau burden in the entorhinal cortex compared to controls. Prior pathological studies of tauopathy in C9or72-related FTD and ALS/FTD [2,3] identified a burden of neurofibrillary tangles that ranged from a mild (in most cases) to a moderate/high likelihood of AD by Braak staging. PART was seen in ~50% of C9or72 expansion carriers. Postmortem analysis of a C9or72 and entorhinal tau PET-positive participant revealed entorhinal tau pathology in the absence of β-amyloid deposition, most consistent with PART. However, tau deposition was not associated with clinical features or psychometric performance.

AV-1451 has been shown to bind neurofibrillary tangles and paired helical filament tau in situ [4]. However, its propensity to bind tau in alternatively modified or aggregated states is not well characterized. The presence of alternative tau species with poor tracer affinity cannot be excluded in C9or72-related disease.

Study limitations include lack of testing for episodic memory or behavioral changes, a relatively small sample size and relatively mild cognitive burden in this cohort. A larger longitudinal study of C9or72 expansion carriers spanning from pure FTD to pure ALS would help address the predictive power of AV-1451 as a biomarker for cognitive impairment in these patients.

**Acknowledgements**

The WU Knight Alzheimer’s Research Imaging Program and the Knight ADRC clinical core are thanked for participant assessments. Ms Marina Platik is thanked for optimizing the immunohistochemistry protocols. Our research participants and their families are also thanked.

**Disclosure of conflicts of interest**

Funding for the study was received from a Washington University McDonell Center grant; National Institutes of Health T32 Training grant NS007205 (Dr Ly); American Academy of Neurology/ ALS Association Clinical Research Training fellowship (Dr Ly); National Institute of Health grant K08NS107621; National Institute of Neurological Disease and Stroke grant P30 NS048056; the Hope Center for Neurodegenerative Disorders (Dr Benzinger); the Barnes-Jewish Hospital Foundation Willman Scholar Fund (Dr Gordon); the American Society for Neuroradiology (Dr Gordon); National Institute of Health grant K01AG053474 (Dr Gordon); National Institute of Health grants P01AG03991, P50AG005681, P01AG026276, UL1TR000448, P30NS098577. Dr Ances is funded by grants R01NR012907, R01NR012657, R01NR014449, 1R01AG057680, 1R01AG052550 from the National Institutes of Health and from the Paula and Rodger O Riney Fund. Dr Miller is funded by grants R01NS078398 and R01NS097816 from the National Institutes of Health. Dr. Miller reports support for clinical studies and is a medical advisor for Biogen, material support and licensing agreement with Ionis Pharmaceuticals, licensing agreement with C2N, and consults a consultant for Cytokinetics. Support from Avid Radiopharmaceuticals (a wholly owned subsidiary of Eli Lilly) included provision of the precursor to AV-1451 and radiochemistry support.

**References**


