TDP-43 Pathology, Cognitive Decline, and Dementia in Old Age

Robert S. Wilson, PhD; Lei Yu, PhD; John Q. Trojanowski, MD, PhD; Er-Yun Chen, MD; Patricia A. Boyle, PhD; David A. Bennett, MD; Julie A. Schneider, MD

IMPORTANCE Cognitive decline is a leading cause of disability and death in old age but its neurobiological bases are not well understood.

OBJECTIVE To test the hypothesis that transactive response DNA-binding protein 43 (TDP-43) is related to late-life cognitive decline.

DESIGN, SETTING, AND PARTICIPANTS Longitudinal clinical-pathologic cohort study involving more than 40 Catholic groups across the United States. A total of 130 older Catholic nuns, priests, and monks underwent annual clinical evaluations, including detailed cognitive testing, for a mean of 10.1 years prior to death. On neuropathologic examination, we collected semiquantitative measures of TDP-43 pathology, density of neuronal neurofibrillary tangles, area occupied by amyloid-beta plaques, and the presence of alpha-synuclein Lewy bodies from multiple brain regions. Gross and microscopic cerebral infarcts and hippocampal sclerosis were also identified.

MAIN OUTCOMES AND MEASURES Annual rate of change in a previously established composite measure of global cognition during a mean of 10.1 years of annual observation before death.

RESULTS Transactive response DNA-binding protein 43 pathology, ranging from sparse to severe, was identified in 46% of participants and was associated with amyloid plaques, tangles, and hippocampal sclerosis but not neocortical Lewy bodies or cerebral infarcts. After controlling for amyloid plaques, tangles, and hippocampal sclerosis, TDP-43 pathology was associated with more rapid cognitive decline and accounted for nearly as much of the variability in rates of global cognitive decline as did tangles. Transactive response DNA-binding protein 43 pathology had a distinct cognitive profile that differed from other neuropathologic processes (related to decline in episodic and working memory but not in other cognitive domains), and it was elevated in those who developed dementia but not in those with mild cognitive impairment.

CONCLUSION AND RELEVANCE The results suggest that TDP-43 is an important brain pathology underlying cognitive decline and dementia in old age.
TDP-43 Pathology, Cognitive Decline, and Dementia in Old Age

Original Investigation Research

TDP-43 pathology, Cognitive Decline, and Dementia in Old Age

Methods

Participants

We used data from persons in the Religious Orders Study, a longitudinal clinical-pathologic investigation of older Catholic nuns, priests, and brothers recruited from more than 40 groups across the United States. Eligibility required age older than 55 years, absence of a prior dementia diagnosis, and agreement to annual clinical evaluations (begun in 1994 and continuing) and organ donation at death. All participants signed an informed consent and anatomic gift act. The project was approved by the institutional review board of Rush University Medical Center.

At the time of these analyses, 539 of 1081 study participants without baseline dementia had died. Of these, 505 (94%) had undergone a brain autopsy, which had been completed in the first consecutive 490, of whom 463 had longitudinal cognitive data. Of these, TDP-43 data had been collected in 130. Compared with the 333 without TDP-43 data, the 130 with TDP-43 had more follow-up (10.1 years vs 8.7 years; χ² = 17.7; P < .001) but did not differ in age, sex, education, global cognition (at baseline or proximate to death), postmortem interval, amyloid plaques, tangles, hippocampal sclerosis, neocortical Lewy bodies, or cerebral infarcts. They died at a mean (SD) age of 88.1 (7.5) years after a mean (SD) of 10.1 (3.1) years of annual cognitive testing. They had completed a mean (SD) of 18.0 (3.3) years of education and 70.8% were women.

Assessment of Cognitive Function

Cognition was assessed annually with a battery of 20 tests that are described in Table 1. To minimize measurement error, we used composite cognitive measures in most analyses. We formed a composite measure of global cognition that used all 20 tests and based in part on previous factor analyses in this and other14,15 cohorts, composite measures of episodic memory (based on 7 tests), semantic memory (4 tests), working memory (4 tests), perceptual speed (2 tests), and visuospatial ability (2 tests). Scores on individual tests were converted to z scores, using the baseline mean and standard deviation, and the z scores were averaged to yield each composite. We also analyzed change in the raw scores of each individual test.

Clinical Evaluation

The annual evaluations also included a medical history and neurological examination.11-13 After each evaluation, an experienced clinician diagnosed dementia and mild cognitive impairment. Classification of dementia required a history of cognitive decline and impairment in at least 2 cognitive domains.16 To maintain uniformity in the diagnostic process, we used an algorithm to rate impairment in 5 cognitive domains (orientation, attention, memory, language, and perception) based on educational level and scores on 11 of the cognitive tests.17 After review of all cognitive data, a neuropsychologist agreed or disagreed with each algorithmic rating and supplied new ratings in the event of disagreements. The diagnosis of mild cognitive impairment required impairment in 1 or more cognitive domains in the absence of dementia.17 On death, all clinical data were reviewed by a board-certified neurologist blinded to all pathologic data and final diagnoses of mild cognitive impairment and dementia were made.

Neuropathologic Examination

The brain was removed a median of 5.5 hours (interquartile range, 5.4 hours) after death, which occurred a median of 7.7 months (interquartile range, 7.0 months) following the last clinical evaluation. One cerebral hemisphere, one cerebellar hemisphere, and the brainstem were fixed in 4% paraformaldehyde for at least 72 hours. The brain was cut coronally into 1-cm slabs and all slabs were examined for gross infarcts. A standard protocol was followed for tissue preservation, tissue sectioning, and quantification of pathologic data by examiners blinded to all clinical data.18,19 We used hematoxylin and eosin to identify microinfarcts (ie, visible on microscopic but not gross inspection) in 9 regions in 1 hemisphere, as previously described.20 In analyses, chronic gross and microscopic infarcts were each treated as present or absent.

Based on prior research,1,21-26 we investigated TDP-43 pathology in 6 brain regions: the amygdala (and periamygdalar region, when available), hippocampus CA1/subiculum, dentate gyrus, entorhinal cortex, midfrontal cortex, and middle temporal cortex. Immunostaining was done on 6-μm sec-
tions using monoclonal antibodies to phosphorylated TDP-43 (pS409/410; 1:100), 27 which stain the pathologically phosphorylated TDP-43 proteins in the inclusions seen in amyotrophic lateral sclerosis, frontotemporal lobar degeneration, and other neurodegenerative diseases but not the normal nuclear TDP-43. Each region of interest was reviewed for the presence, severity, and location of TDP-43 cytoplasmic inclusions (both neuronal and glial) and was rated on a 6-point scale based on the number of inclusions in a 0.25-mm² area of greatest density within that region (none, sparse [1-2 inclusions], sparse to moderate [3-5 inclusions], moderate [6-12 inclusions], moderate to severe [13-19 inclusions], and severe [20 or more inclusions]) (Figure 1).

An antipaired helical filaments-tau antibody clone AT8 (ThermoScientific; 1:2000) and computer-assisted sampling 28 were used to measure density of tau-immunoreactive neurofibrillary tangles in at least 2 sections from 8 limbic and neocortical regions (entorhinal cortex, CA1/subiculum, anterior cingulate cortex, dorsolateral prefrontal cortex, superior frontal cortex, inferior temporal cortex, inferior parietal cortex, and primary visual cortex). The raw scores in each section and region were averaged to yield a composite measure of tangle density per square millimeter, as previously described. 26

Beta-amyloid-immunoreactive plaques were assessed in the 8 regions examined for tau using a monoclonal antibody (1:50; beta-amyloid, Clone 6F/3D, Dako) with diaminobenzidine as the reporter, with 2.5% nickel sulfate to enhance contrast. Computer-assisted sampling and image analysis were used to quantify the percentage of each area occupied by beta-amyloid–immunoreactive pixels. Regional measures were averaged to yield a composite measure of amyloid burden. 28

Lewy bodies were identified in the substantia nigra, 2 limbic sites (entorhinal cortex and anterior cingulate cortex), and 3 neocortical sites (midfrontal cortex, superior or middle temporal cortex, and inferior parietal cortex) using a monoclonal antibody to alpha-synuclein (Zymed LB509; 1:50). 18 We used a modified version 29, 30 of the staging criteria of McKeith et al 31 to classify Lewy body disease as nigral, limbic, or neocortical. Neocortical disease required Lewy bodies in the frontal, temporal, or parietal cortex and was usually accompanied by nigral and limbic Lewy bodies.

### Statistical Analysis

To assess agreement among TDP-43 raters, we calculated the component of variance owing to rater as a fraction of the total variance for each brain region. The dimensionality of the re-

| Cognitive Measure | Function Assessed | Items |  | Evaluation |  |  |  |  |  |  |  |  |  |  |  |  |  |
|--------------------|-------------------|------|---|------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Logical memory     |                   |      |   | Baseline   | Last | Annual Change* | Baseline | Last | Annual Change* | Baseline | Last | Annual Change* | Baseline | Last | Annual Change* | Baseline | Last | Annual Change* | Baseline | Last | Annual Change* |
| ia Episodic memory |                   | 25   |   | 11.8 (3.7) | 8.6 (5.7) | −0.078 | 0.13 | <.001 |
| ila Episodic memory|                   | 25   |   | 10.0 (4.0) | 7.8 (5.5) | −0.061 | 0.12 | <.001 |
| Story recall       | Episodic memory   | 12   |   | 9.8 (1.8)  | 7.2 (3.5) | −0.123 | 0.15 | <.001 |
| Delayed            | Episodic memory   | 12   |   | 9.2 (2.2)  | 6.5 (3.8) | −0.108 | 0.15 | <.001 |
| Word list          | Episodic memory   | 30   |   | 17.5 (4.0) | 14.0 (7.0) | −0.091 | 0.16 | <.001 |
| Memory             | Episodic memory   | 10   |   | 5.4 (2.1)  | 3.8 (3.0) | −0.070 | 0.13 | <.001 |
| List recall        | Episodic memory   | 10   |   | 9.6 (1.2)  | 8.1 (3.1) | −0.110 | 0.23 | <.001 |
| List recognition   | Episodic memory   | 15   |   | 10.6 (3.3) | 8.7 (4.5) | −0.053 | 0.11 | <.001 |
| Vocabulary         | Semantic memory   | 20   |   | 18.2 (2.4) | 17.0 (4.0) | −0.036 | 0.15 | <.001 |
| Reading            | Semantic memory   | 20   |   | 13.5 (8.6) | 21.1 (11.9) | −0.127 | 0.12 | <.001 |
| Verbal fluency     | Semantic memory   | 10   |   | 18.0 (1.9) | 15.7 (4.5) | −0.101 | 0.16 | <.001 |
| Boston Naming Test | Semantic memory   | 12   |   | 8.4 (2.0)  | 7.1 (2.8) | −0.046 | 0.13 | <.001 |
| Digit span         | Working memory    | 12   |   | 6.1 (1.9)  | 4.5 (2.6) | −0.066 | 0.12 | <.001 |
| Digit ordering     | Working memory    | 16   |   | 6.8 (2.7)  | 5.5 (2.5) | −0.051 | 0.09 | <.001 |
| Alpha span         | Working memory    | 14   |   | 4.8 (1.6)  | 2.8 (1.9) | −0.099 | 0.11 | <.001 |
| Symbol Digit Modalities Test | Perceptual speed | 110  |   | 38.4 (10.0) | 23.6 (14.8) | −0.125 | 0.11 | <.001 |
| No. comparison     | Perceptual speed  | 48   |   | 24.2 (6.5) | 16.3 (8.9) | −0.106 | 0.12 | <.001 |
| Line orientation   | Visuospatial ability | 15    | 9.7 (3.2)  | 7.7 (3.8) | −0.066 | 0.10 | <.001 |
| Standard Progressive Matrices | Visuospatial ability | 17 | 9.8 (3.2)  | 7.8 (3.4) | −0.052 | 0.09 | <.001 |
| Mini-Mental State Examination | Global cognition | 30  | 28.3 (1.6) | 21.0 (9.4) | −0.215 | 0.030 | <.001 |

Abbreviations: NA, not available; SE, standard error.

* From 20 separate mixed-effects models adjusted for age at death.
regional TDP-43 measures was assessed in a principal-components analysis. We used the Loess procedure to determine whether TDP-43 had a linear relation to cognitive slope and then used linear mixed-effects models to estimate the association of TDP-43 with the level of global cognition and annual rate of change. Each model included terms for time (in years from death), age at death, TDP-43, and the interactions of time with age and TDP-43. We repeated the analysis with additional pathologic measures and their interactions with time and then with multiple cognitive outcomes. Clinical diagnosis proximate to death, with a mild cognitive impairment reference group contrasted with no cognitive impairment and dementia groups, was regressed on TDP-43 in a polytomous logit model adjusted for age at death and AD pathology (but not hippocampal sclerosis, which was present in only 1 person without dementia).

**Results**

As shown in Table 2, the regional measures of TDP-43 were positively skewed, with no TDP-43 pathology in 54% and levels ranging from sparse to severe in the remaining 46%. Transactive response DNA-binding protein 43 pathology was most common in the amygdala (45%), less common in the entorhinal cortex (22%) and hippocampus (16%-21%), and least common in the neocortex (5%-10%). None of those with neocortical TDP-43 pathology had degeneration of the frontal or temporal lobes plus layer 2 spongiform changes compatible with a diagnosis of frontotemporal lobar degeneration with TDP-43 inclusions. The intraclass correlation coefficients of the regional measures, based on independent ratings of an approximate 8% subset of slides by 3 individuals, indicate good interrater reliability (Table 2). In a principal-components analysis, the regional TDP-43 measures loaded on a single factor that accounted for 63.3% of the variance (Table 2). Therefore, we summed the regional measures to create an index of the severity and extent of TDP-43 pathology (mean [SD], 3.00 [5.19]; skewness, 2.00; range, 0-21). The 60 persons (46%) with at least some evidence of pathology on this measure were older at death than unaffected persons (eTable 1 in Supplement).

Beta-amyloid plaque burden ranged from 0 to 14.87 (n = 130; mean [SD], 2.72 [2.89]; skewness, 1.23) and tau tangle density ranged from 0 to 61.55 (n = 130; mean [SD], 5.05 [7.46]; skewness, 4.10). Also, 39.2% had at least 1 chronic gross infarct, 28.5% had at least 1 chronic microinfarct, 7.7% had neocortical Lewy bodies, and 9.2% had hippocampal sclerosis. The presence of TDP-43 pathology was associated with higher amyloid plaque burden, tangle density, and likelihood of hippocampal sclerosis but not with likelihood of gross infarcts, microinfarcts, or neocortical Lewy bodies (eTable 2 in Supplement).

**TDP-43 and Global Cognitive Decline**

The top panel of Figure 2, which shows the rate of global cognitive decline plotted by level of TDP-43 fit with a Loess,

---

**Table 2. Descriptive Information on Regional Measures of TDP-43**

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Frequency of TDP-43 Scores</th>
<th>Intraclass Correlation a</th>
<th>Factor Loading b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amygdala</td>
<td>130</td>
<td>1.21 (1.73)</td>
<td>0.90</td>
</tr>
<tr>
<td>Entorhinal cortex</td>
<td>128</td>
<td>0.61 (1.33)</td>
<td>0.97</td>
</tr>
<tr>
<td>CA1/subiculum</td>
<td>127</td>
<td>0.50 (1.05)</td>
<td>0.84</td>
</tr>
<tr>
<td>Dentate gyrus</td>
<td>125</td>
<td>0.40 (1.01)</td>
<td>0.94</td>
</tr>
<tr>
<td>Middle temporal gyrus</td>
<td>129</td>
<td>0.23 (0.80)</td>
<td>0.89</td>
</tr>
<tr>
<td>Midfrontal gyrus</td>
<td>128</td>
<td>0.09 (0.51)</td>
<td>0.96</td>
</tr>
</tbody>
</table>

Abbreviation: TDP-43, transactive response DNA-binding protein 43.

a. Indicator of interrater reliability.

b. From a principal-components analysis.
suggests that the association of TDP-43 with the rate of cognitive decline is approximately linear and supports the use of linear mixed-effects models. In the initial analysis, there was a mean decline of 0.073 units per year in the global cognitive measure (standard error [SE], 0.012; \( P < .001 \)). Higher levels of TDP-43 inclusions were associated with lower levels of global cognition (estimate, −0.090; SE, 0.021; \( P < .001 \)) and more rapid global cognitive decline (estimate, −0.010; SE, 0.002; \( P < .001 \)).

Because TDP-43 was associated with amyloid plaques, tangles, and hippocampal sclerosis, we repeated the analysis with terms added for these pathologic measures. In this model, TDP-43 (estimate, −0.006; SE, 0.002; \( P = .007 \)) and tangles (estimate, −0.007; SE, 0.002; \( P < .001 \)) were each related to more rapid cognitive decline with no association for amyloid (estimate, −0.002; SE, 0.004; \( P = .60 \)) or hippocampal sclerosis (estimate, −0.048; SE, 0.037; \( P = .20 \)). The bottom panel of Figure 2, which is based on this analysis, suggests a dose-response relationship with higher levels of TDP-43 pathology associated with increasingly rapid cognitive decline.

In further analyses, we compared the impact of TDP-43 and tangles on cognitive aging. Relative to a model adjusted for age at death, TDP-43 accounted for an additional 21% of the variance in rates of cognitive decline vs 26% for tangles. With tangles in the model, TDP-43 accounted for an additional 12% of the variance vs 16% for tangles with TDP-43 in the model.

TDP-43 and Decline in Cognitive Domains
To determine whether TDP-43 was related to decline in some cognitive functions but not others, we assessed change in 5 different cognitive domains (Table 3). A higher level of TDP-43 pathology was associated with more rapid decline in episodic memory and working memory but not with decline in other domains. In contrast, tangle density was associated with decline in all domains, amyloid plaque burden was not related to decline in any domain, and hippocampal sclerosis was associated with decline in semantic memory but not other domains.

We conducted additional analyses with the individual tests as outcomes instead of composite measures. For each test, Table 1 shows the mean score at baseline, the mean score proximate to death, and the estimated annual rate of change. In the analyses (Table 2 in Supplement), TDP-43 was related to decline on all 7 episodic memory tests, the Boston Naming Test, Digit Span Forward, and the Mini-Mental State Examination. Tangle density was associated with decline on 15 tests, amyloid plaque burden with decline on 2 tests (immediate story recall and Boston Naming Test), and hippocampal sclerosis associated with decline in semantic memory but not other domains.
TDP-43 and Clinical Diagnoses

Proximate to death, 38 participants (29%) had no evidence of cognitive impairment, 39 (30%) had mild cognitive impairment, and 53 (41%) had dementia. To determine whether TDP-43 was differentially related to cognition along the spectrum from intact function to dementia, we examined its relation to clinical diagnosis. In an analysis that included terms for age at death, amyloid plaques, and tangles, a higher level of TDP-43 pathology was associated with a higher likelihood of dementia relative to no cognitive impairment ($\chi^2 = 5.35$, $P = .02$) but not with likelihood of mild cognitive impairment relative to no cognitive impairment ($\chi^2 = 0.17$, $P = .68$). By contrast, tangles had a nearly significant association with mild cognitive impairment relative to no cognitive impairment ($\chi^2 = 2.97$, $P = .09$) but did not differentiate mild cognitive impairment from dementia ($\chi^2 = 0.28$, $P = .59$), and amyloid plaques were not related to diagnosis.

Discussion

A cohort of 130 older persons without dementia at study entry underwent annual cognitive testing for a mean of about 10 years during which more than 40% developed dementia. On neuropathologic examination, TDP-43 pathology was identified in nearly half of the participants, and it accounted for nearly as much of the variability in the rates of cognitive decline as did AD pathology. The findings suggest that TDP-43 is an important pathology underlying late-life cognitive decline and dementia.

In previous research, TDP-43 pathology has been associated with a lower level of global cognition and higher likelihood of dementia. The present results extend these observations in 2 important ways. First, the association of TDP-43 with lower levels of global cognition persisted after controlling for age, AD pathology, and hippocampal sclerosis, supporting the idea that its association with cognitive impairment is relatively independent of these other pathologic processes. Second, after controlling for this association, age, and other pathologies, TDP-43 was associated with more rapid cognitive decline. That it accounted for nearly as much of the variability in cognitive decline as neurofibrillary tangles suggests that TDP-43 pathology plays a substantial role in late-life loss of cognition.

The manner in which TDP-43 pathology leads to impaired neuronal function is not known. Multiple pathways activated by TDP-43 aggregation may be involved, but the downstream consequences of TDP-43 phosphorylation, aggregation, cleavage, mislocalization, and clearance from the nucleus are still unclear. The clearance of normal TDP-43 from the nucleus may represent a loss of normal TDP-43 that leads to neurodegeneration, or, alternatively, the retention of TDP-43 in cytoplasmic aggregates could sequester diverse RNAs and thereby lead to neurodegeneration through a toxic gain of function.

Whether TDP-43 pathology has a characteristic cognitive profile is not known. To our knowledge, few TDP-43 studies have assessed multiple cognitive functions and results have been inconsistent. For example, TDP-43 was associated with impairment of semantic memory but not episodic memory in one study, whereas another study reported the opposite pattern. This inconsistency may reflect several factors including reliance on cross-sectional cognitive data and the potentially confounding effects of other pathologic processes on cognition. In the present study, TDP-43 was associated with impairment and decline in multiple cognitive domains. However, after adjustment for AD pathology and hippocampal sclerosis, TDP-43 pathology was related to decline in episodic and working memory but not to decline in other cognitive domains. The cognitive profile associated with TDP-43 pathology differed from the cognitive profiles associated with AD pathology and hippocampal sclerosis. Consistent with prior research, TDP-43 pathology was most common in the medial temporal lobe, which may account for its robust association with episodic memory dysfunction.

In this cohort, TDP-43 pathology was increased in persons with dementia but not in those with mild cognitive impairment. This suggests that TDP-43 may be more strongly related to progression of cognitive symptoms than to their initial development, or that it is a separate age-related process that is age shifted, occurring in most instances after the onset of other age-related neurodegenerative processes. In contrast, the pathologic processes traditionally associated with late-life dementia (eg, tangles, Lewy bodies, and cerebral infarcts) appear to account for more of the variability in incipient cognitive decline than in later acceleration of decline.

Study strengths and limitations should be noted. A uniform clinical evaluation and established criteria were used to clinically classify individuals. Participation in annual follow-up and autopsy was high, minimizing risk for bias owing to selective attrition. The availability of a mean of approximately 10 years of annual cognitive assessments with psychometrically established measures allowed us to reliably estimate individual trajectories of cognitive change. Because of the selected nature and relatively small size of the group studied, it will be important to replicate these findings. In addition, use of a nonphosphorylated epitope rather than a phosphorylated antibody probably resulted in underestimation of the burden of synucleinopathy.
Obtained funding: Wilson, Bennett, Schneider. Administrative, technical, or material support: Wilson, Trojanowski, Chen, Boyle, Bennett, Schneider. Study supervision: Wilson, Trojanowski, Bennett, Schneider.

Conflict of Interest Disclosures: None reported.

Funding/Support: This research was supported by National Institute on Aging grants RO1AG24210, P30AG10161, RO1AG51891, RO1AG10124, and RO1AG23953, as well as by the Illinois Department of Public Health.

Role of the Sponsor: The funding organizations had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Additional Contributions: We thank the many Catholic clergy members who have participated in the Religious Orders Study; Karen Slish, MS, for overall coordination of pathologic data collection; Sabrani Monalid, MS, for immunohistochromatic staining; Virginia Kriho, MS, for TDP-43 data collection; Traci Colvin, MPH, for coordinating the clinical study; Woojoeng Bang, MS, for statistical programming; and John Gibbons, MS, and Greg Klein, MS, for data management.

REFERENCES