Pathological 43-kDa Transactivation Response DNA-Binding Protein in Older Adults With and Without Severe Mental Illness

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Background: Major psychiatric diseases such as schizophrenia and mood disorders have not been linked to a specific pathology, but their clinical features overlap with some aspects of the behavioral variant of frontotemporal lobar degeneration. Although the significance of pathological 43-kDa (transactivation response) DNA-binding protein (TDP-43) for frontotemporal lobar degeneration was appreciated only recently, the prevalence of TDP-43 pathology in patients with severe mental illness vs controls has not been systematically addressed.

Objective: To examine patients with chronic psychiatric diseases, mainly schizophrenia, for evidence of neurodegenerative TDP-43 pathology in comparison with controls.

Design: Prospective longitudinal clinical evaluation and retrospective medical record review, immunohistochemical identification of pathological TDP-43 in the central nervous system, and genotyping for gene alterations known to cause TDP-43 proteinopathies including the TDP-43 (*TARDBP*) and progranulin (*GRN*) genes.

Setting: University health system.

Participants: One hundred fifty-one subjects including 91 patients with severe mental illness (mainly schizophrenia) and 60 controls.

Main Outcome Measures: Clinical medical record review, neuronal and glial TDP-43 pathology, and *TARDP* and *GRN* genotyping status.

Results: Significant TDP-43 pathology in the amygdala/periamygdaloid region or the hippocampus/transentorhinal cortex was absent in both groups in subjects younger than 65 years but present in elderly subjects (29% [25 of 86] of the psychiatric patients and 29% [10 of 34] of control subjects). Twenty-three percent (8 of 35) of the positive cases showed significant TDP-43 pathology in extended brain scans. There were no evident differences between the 2 groups in the frequency, degree, or morphological pattern of TDP-43 pathology. The latter included (1) subpial and subependymal, (2) focal, or (3) diffuse lesions in deep brain parenchyma and (4) perivascular pathology. A new *GRN* variant of unknown significance (c.620T>C, p.Met207Thr) was found in 1 patient with schizophrenia with TDP-43 pathology. No known *TARDBP* mutations or other variants were found in any of the subjects studied herein.

Conclusions: The similar findings of TDP-43 pathology in elderly patients with severe mental illness and controls suggest common age-dependent TDP-43 changes in limbic brain areas that may signify that these regions are affected early in the course of a cerebral TDP-43 multisystem proteinopathy. Finally, our data provide an age-related baseline for the development of whole-brain pathological TDP-43 evolution schemata.

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THE PATHOLOGICAL SUBstrates of severe mental illnesses (SMI) have been debated without consensus among experts in the field ever since the time of Kraepelin[1] and Bleuler.[2] Although many studies have shown various structural and functional changes indicative of subcortical and cortical brain pathology in schizophrenia, the underlying cellular neuropathology of schizophrenia, as well as for mood disorders, remains to be elucidated. Indeed, there continues to be ongoing debate on the relative contributions of neurodevelopmental vs neurodegenerative pathophysiologies of schizophrenia and other psychotic disorders.[3-5] Studies of neurodegenerative pathology such as tau or β-amyloid lesions have been reported in schizophrenia with conflicting results. The consensus is that schizophrenia is not mediated by α-synuclein–, prion-, tau-, or β-amyloid–induced neurodegeneration as

occurs in Lewy body disease, prion disorders, or Alzheimer disease (AD).[4,6-10]

Discoveries of new neurodegenerative disease pathologies offer opportunities to determine if they may play a role in schizophrenia. For example, recently, 43–kDa transactivation response DNA-binding protein (TDP-43) was discovered to be the disease protein in frontotemporal lobar degeneration with ubiquitin-positive and tau- and α-synuclein–negative inclusions (FTLD-U), amyotrophic lateral sclerosis, and FTLD-U combined with amyotrophic lateral sclerosis. This led to the recognition of a novel multisystem clinicopathological spectrum disorder, ie, TDP-43 proteinopathies[11-13] by analogy with other neurodegenerative diseases such as tauopathies or α-synucleinopathies, and FTLD-U is now termed *FTLD-TDP*.[14]

Although the significance of pathological TDP-43 for human disease has been appreciated in the last 3 years,[12] the prevalence of TDP-43 pathology in normal controls has not been definitely determined yet. However, it is plausible that TDP-43 lesions might occur at a prevalence in controls similar to those noted for tau, β-amyloid, and α-synuclein depositions in controls.[15-17] The relevance of the question as to the presence of TDP-43 pathology in controls is emphasized by the fact that the available studies on pathological TDP-43 in human diseases are largely restricted to late- or end-stage disease findings and relatively few control subjects have been examined for the presence of TDP-43 pathology. Indeed, there is a relative lack of information about TDP-43 pathology in the early phase of the disease course when the subjects are asymptomatic, or show mild signs of cognitive dysfunction, or when behavioral or memory changes are present that could reflect prodromal disease.[12]

Data on TDP-43 pathology in schizophrenia or mood disorders are scant and based on limited assessment of a few brain regions. In fact, 1 small study on schizophrenia was negative for pathological TDP-43,[18] and in another study, single cases of late-onset psychosis were reported to be associated with altered TDP-43 nuclear staining.[19] Further, a few cases of patients with pathological TDP-43–positive young-onset frontotemporal dementia (FTD) with psychosis as the initial feature have been reported.[20] Moreover, the available studies on controls have been limited and heterogeneous but have reported little or minimal TDP-43 pathology.[12,19,21-32] Given that the behavioral variant of FTD may overlap with some aspects of chronic schizophrenia and the paucity of data on TDP-43 pathology in patients with chronic psychiatric diseases, including schizophrenia, as well as in controls, we examined a large cohort of patients with chronic SMI, mainly schizophrenia, to determine if they show central nervous system (CNS) accumulations of pathological TDP-43 and compared these results with TDP-43 studies in normal controls. In addition, we genotyped patients with schizophrenia for gene alterations that are known to cause TDP-43 proteinopathies, including the TDP-43 (*TARDBP*) and progranulin (*GRN*) genes.

Significant TDP-43 pathology in the amygdala/periamygdaloid region or the hippocampus/transentorhinal cortex was absent in both groups in subjects younger than 65 years but present in elderly subjects (29% [25 of 86] of the psychiatric patients and 29% [10 of 34] of control subjects). TDP-43–linked neurodegeneration exhibited 4 pathological lesion patterns including (1) subpial or subependymal, (2) focal, or (3) diffuse lesions in deep brain parenchyma as well as (4) perivascular pathology. Overall, there were no apparent differences in the frequency, degree, or pattern of pathology between both the SMI and control groups. Twenty-three percent (8 of 35) of these cases showed significant TDP-43 pathology in extended brains scans. The TDP-43–positive group was of significantly higher age at death compared with the TDP-43–negative subjects (85 vs 74.5 years; $P < .001$). Although no diagnostic brain topographical algorithms for TDP-43 pathology in TDP-43 proteinopathies have been developed yet, these results presented herein may indicate that TDP-43 lesions develop in limbic brain areas early in the course of cerebral neurodegenerative TDP-43 diseases or proteinopathies. However, this view requires further study and these data provide an age-related baseline for the prevalence of TDP-43 in controls, which will be important for developing whole-brain evolution schemes for TDP-43 pathology in neurodegenerative TDP-43 proteinopathies.

METHODS

STUDY SUBJECTS

Individuals who underwent autopsy in the Center for Neurodegenerative Disease Research at the University of Pennsylvania from 1985 to 2009 were enrolled. These included (1) patients with a chronic SMI, mainly schizophrenia, but also cases of a schizoaffective or pure affective disorder; (2) control subjects without any known major psychiatric or neurologic condition. The patients with SMI scrutinized herein were longitudinally followed up by University of Pennsylvania investigators at state hospitals in the Commonwealth of Pennsylvania.[33] All patients were prospectively recruited into the study and clinically assessed, and retrospective clinical medical record review was performed for further historical and clinical data.[33,34] Informed consent for autopsy was obtained in all cases from the patient's family or legal representative in accordance with the Commonwealth of Pennsylvania law as well as protocols approved by the University of Pennsylvania institutional review boards.

To screen for TDP-43 pathology, the hippocampus/transentorhinal cortex and/or amygdala/periamygdaloid region were stained for TDP-43. We chose to stain these areas because they are among the CNS regions most consistently affected by accumulations of TDP-43 pathologies in FTLD-U.[13] Those cases with evident TDP-43 pathology were then subjected to a more extensive immunohistochemical CNS examination, including frontal and superior-midtemporal cortex, cingulate, deep brain nuclei (striatum/lentiform nucleus, thalamus), and rhombencephalon (substantia nigra, cerebellum, medulla oblongata), to assess other areas commonly affected by TDP-43 pathology.[11,13] In a subset of cases, various other mesocortical and neocortical brain areas in the frontal (ie, orbitofrontal), parietal (ie, angular), and occipital (ie, visual) cortex were also examined (see later).

IMMUNOHISTOCHEMICAL EXAMINATION

All cases were fully examined by routine diagnostic techniques as described previously.[11,13,35,36] Briefly, small blocks of freshly dissected tissues from multiple CNS areas were fixed

in 10% neutral buffered formalin or 70% ethanol with 150mM sodium chloride, paraffin embedded, and cut into 6-μm sections. Sections were subjected to immunohistochemical examination using (1) the avidin-biotin complex detection method (Vectastatin ABC kit; Vector Laboratories, Burlingame, California) or (2) BioGenex Super Sensitive Detection System Kit (BioGenex Laboratories, San Ramon, California) with 3,3-diaminobenzidine as the chromogen. The following primary antibodies were used: mouse anti–paired helical filament-1 monoclonal antibody (mAb) (a gift of Peter Davies, PhD; 1:1000), mouse antiubiquitin mAb (1510; Chemicon, Temecula, California; 1:100 000), rabbit polyclonal anti–TDP-43 (Proteintech Group, Chicago, Illinois; 1:4500), rat antiphosphorylated TDP-43 mAb (S409/410[36]; 1:1000), mouse anti–TDP-43 mAb (TDP 171; generated in the Center for Neurodegenerative Disease Research, Philadelphia, Pennsylvania; 1:50 000), mouse anti–α-synuclein mAb (Syn303; generated in the Center for Neurodegenerative Disease Research; 1:4000), mouse anti–human leucocyte antigen mAb (DakoCytomation, Glostrup, Denmark; 1:5000), rabbit polyclonal anti–glial fibrillary acidic protein antibody (DakoCytomation; 1:5000), mouse anti–microtubule associated protein 2 (M12) mAb (generated in the Center for Neurodegenerative Disease Research; 1:1), and rabbit polyclonal anti–fused in sarcoma (FUS) antibody (Sigma-Aldrich, St Louis, Missouri; 1:500). Sections stained for ubiquitin, TDP-43, and human leucocyte antigen, M12, and FUS were pretreated by boiling in citrate antigen unmasking solution (Vector Laboratories; 1:100) using a microwave, and those stained for α-synuclein were pretreated with 80% formic acid. Double-labeling immunofluorescence immunohistochemical examination using Alexa Fluor 488– and 594–conjugated secondary antibodies (Molecular Probes, Eugene, Oregon) was performed as previously described.[11,35] TDP-43 inclusions were assessed based on morphologies and distribution in a given brain area as described elsewhere.[13,27] Positive controls were human disease CNS tissue sections with known pathological reactivity to the antibody in question, and they were included in every immunohistochemical staining procedure as described previously.[11,13,35,36] Further, normal nuclear staining in unaffected regions of CNS sections served as internal controls for each slide. In addition, immunohistochemical staining of selected cases found to show TDP-43 immunoreactivity was performed with the omission of the primary rabbit polyclonal anti–TDP-43 (Proteintech Group) to investigate the specificity of recognizing actual TDP-43 pathology. Digital images of immunohistochemical examination and immunofluorescence were obtained using an Olympus BX 51 (Tokyo, Japan) microscope using a digital camera DP71 (Olympus, Orangeburg, New York) and DP manager (Olympus).

EVALUATION OF PATHOLOGY

TDP-43 pathology was rated on a 5-point ordinal scale (0, none; 1, rare/minor; 2, mild; 3, moderate; 4, severe/numerous) by 3 of us (F.G., J.L.R., and J.Q.T.). For the purpose of this study, TDP-43 pathology was considered to be "significant" when a grade of 3 or 4 was assigned. We chose the assessment of pathology by means of an ordinal scale rather than by using numeric image analysis-based quantification tools, because the former acknowledges the sequential nature of stages of increasing severity, ultimately corresponding to a spread of pathology throughout a given section or the brain as shown for all major neurodegenerative diseases. In fact, ordinal data provide information about a relation of severity stages rather than being a measurement acknowledging that 1 stage follows continuously into the other, which, therefore, represent sequential classes rather than values on a numerical scale.

GENETIC ANALYSES

Genomic DNA was extracted from brain tissue using standard methods (Qiagen Inc, Valencia, California). The coding region of the *TARDBP* (exons 2-6) and *GRN* (exons 1-13, with exon 1 representing the 5′ untranslated region referred to in previous publications as exon 0) genes encompassing 50 to 200 base pairs of adjacent intronic sequence were bidirectionally sequenced as previously described.[37,38] Briefly, amplification reactions (50 μL) were performed with 100-ng DNA using AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, California) and 200nM (final concentration) of each primer were used (eTable, http://www.archneurol.com). Sequencing was performed by Agencourt Bioscience Corporation (Beverly, Massachusetts). Results were analyzed using Mutation Surveyor software (SoftGenetics LLC, State College, Pennsylvania).

STATISTICAL ANALYSES

The data were analyzed using SPSS 16.0 for Windows (SPSS, Inc, Chicago). The "average" (and "spread") of data on patient characteristics was estimated by calculating the median (and 25th-75th percentiles). For group comparison, the Mann-Whitney *U* test was used. Contingency tables were analyzed with the χ^2 test (or Fisher exact test). The significance level for all comparisons was set at .01 rather than the usual .05 because multiple tests were done. All statistical tests applied were 2-sided.

RESULTS

STUDY SUBJECTS' CLINICAL CHARACTERISTICS AND GENOTYPING FINDINGS

We examined 151 subjects including 91 patients with chronic SMI and 60 controls. The study subject characteristics are summarized in **Table 1**. Schizophrenia was present in 72 cases, schizophrenia with an additional affective component (depression or a bipolar disorder) was recorded in 11 cases, and schizophrenia with anxiety, in 1. Four patients showed an isolated mood disorder. One subject showed cognitive, motor, and behavioral features resembling qualities of an "autistic savant disorder." Another 3 subjects ("gray cases") showed milder clinical features, ie, cognitive (n=2) or predominantly psychiatric (n=1) issues that were not considered diagnostic for a definite disease; these features included both patients' complaints and physician-assessed signs and were not considered exclusionary for this study. Hence, the 2 subjects with the mild cognitive issues were included in the control group (and referred to as "mild cognitive impairment" without applying formal diagnostic criteria). Further, the patient with the predominant psychiatric features was put in the SMI group. There was a variable presence of deterioration in functional and cognitive abilities/dementia and, less commonly, parkinsonism. Based on the whole study cohort, the SMI group was of significantly higher age at death as compared with the control group (Table 1). However, when focusing only on the subjects 65 years and older, there was no significant difference in age at death between the SMI and control groups (median, 80.0 years [range, 75.0-86.0 years]

Table 1. Summary of Study Subjects' Characteristics

Diagnostic Group	No. of Subjects (Male/Female, %)	Age at Onset, y, Median (IQR)	Age at Death, y, Median (IQR)
Subjects with psychiatric disease	91 (38.5/61.5)	24.0 (20.0-30.0)	80.0 (75.0-86.0)
Controls	60 (48.3/51.7)		68.0 (57.5-82.5)
P value	.23[a]		<.001[b]

Abbreviation: IQR, interquartile range.
[a] χ^2 Test.
[b] Mann-Whitney *U* test.

Table 2. Frequency of TDP-43 Pathology in Total Study Cohort and Subjects 65 Years and Older[a]

	No. (%)			No. (%) ≥65 y		
TDP-43 Pathology	SMI (n=91)	Controls (n=60)	*P* Value[b]	SMI (n=86)	Controls (n=34)	*P* Value[b]
Significant (moderate/severe)	25 (27.5)	10 (16.7)	.12	25 (29.1)	10 (29.4)	.97
Mild	9 (9.9)	2 (3.3)	.72	9 (10.5)	2 (5.9)	.73

Abbreviations: SMI, severe mental illness; TDP-43, 43-kDa transactivation response DNA-binding protein.
[a] Rare/minor TDP-43 pathology (including single dystrophic neurites or cytoplasmic inclusions) was found in an additional more than 20% of both the SMI and control groups, almost exclusively in elderly subjects.
[b] χ^2 Test (or Fisher Exact test).

vs median, 77.0 years [range, 70.8-89.2 years]; *P*=.51) allowing for a direct comparison of TDP-43 between these groups.

TDP-43 MICROSCOPIC FINDINGS

Tissue was available and examined for 137 hippocampi/transentorhinal cortices (89 subjects with SMI; 48 controls) and 147 amygdalae/periamygdaloid regions (91 subjects with SMI; 57 controls). Control experiments with the omission of the primary rabbit polyclonal anti–TDP-43 antibody in selected positive cases did not reveal TDP-43 pathology (data not shown). Significant (moderate or severe) and mild TDP-43 pathology was found in only elderly subjects, ie, those 65 years and older. In fact, TDP-43 pathology comprising (1) neuronal and glial cytoplasmic inclusions, (2) pathological cellular processes (dystrophic cellular processes or axonal swellings), (3) roundish neuropil grains, (4) and diffuse, punctuate or dotlike cytoplasmic staining ("preinclusions"), in combination with the absence of nuclear TDP-43 immunoreactivity, and (5) rarely, neuronal intranuclear inclusions were found in a total of 35 study subjects (29.2% of the cohort 65 years and older [n=120] and 23.2% of total cohort [n=151]) (**Table 2**). Moreover, of those cases with significant pathology, 8 psychiatric patients (22.9%) showed significant TDP-43 pathology in the extended brain scan. Indeed, some degree of TDP-43 pathology was present in the brainstem and basal ganglia (**Table 3**). For the cortex, TDP-43 pathology was most pronounced in the amygdala and allocortex but less in the mesocortex or neocortex. The TDP-43 findings of the 35 cases with significant pathology in the (peri)amygdala and/or hippocampus/transentorhinal cortex are depicted in Table 3. For the brain areas not shown on Table 3, mild mesocortical TDP-43 pathology (ie, orbitofrontal cortex) and severe insular cortex TDP-43 pathology were present in 1 case each with severe CA1-subiculum pathology. Significant neocortical TDP-43 pathology was infrequent for the temporal and frontal cortex; additional neocortical brain areas in the parietal and occipital lobe examined in a subset of cases showed similar findings.

Because there were overall no apparent differences in the frequency, degree, or morphological pattern of pathology between the SMI and control groups, the results are presented as a whole in the following summary. Briefly, the brain areas with significant TDP-43 pathology showed morphological features that were sufficient to delineate 4 morphological groups or patterns including subpial/subependymal, diffuse, and focal lesions as well as perivascular pathology, which are described in more detail later. Overall, both the diffuse and focal types of TDP-43 pathology appeared to be more frequent in the gray than in the white matter, and conversely, the perivascular TDP-43 pathology was more common in white than gray matter. Subpial/subependymal pathology was present in both of these compartments. The latter and the perivascular type of TDP-43 pathology were often found in areas that also showed the presence of corpora amylacea. TDP-43 immunoreactivity in the tissue matrix, wherein corpora amylacea were embedded, was variably present and is of uncertain significance.

Subpial/Subependymal TDP-43 Pathology

This morphological pattern was characterized by pathology mainly located at the brain surfaces including both the internal surface, ie, the subependymal location, and external surface, ie, subpial areas (**Figure 1** and eFigure 1). These were present as localized foci or as bandlike swaths of dystrophic cellular processes and cells devoid of the endogenous nuclear staining coupled with diffuse or granular cytoplasmic TDP-43 staining. This was frequently found at internal surfaces immediately subjacent to the ventricular system, ie, subependymal, but also subjacent to pial surfaces (predominantly) in the molecular layer of the cortex.

Focal TDP-43 Pathology

Herein, we use the term *focal pathology* to denote single, small foci of pathology consisting of groups of cells with cytoplasmic pathology or the presence of abnormal cellular processes in deeper regions of the brain parenchyma, and it does not refer to pathology found subjacent to internal or external brain surfaces or in a perivascular localization. Sometimes, single cells consisting of a nucleus devoid of TDP-43 staining were present combined with a weak, diffuse, punctuate or dotlike, or virtually absent cytoplasmic TDP-43, and cell loss was not obvious in these cases (**Figure 2**).

Table 3. Whole-Brain TDP-43 Pathology in Chronic Psychotic Illness and the Controls 65 Years and Older

		ALL						
	COR	PAL		ARC			TRA	
Case No./ Diagnosis	AMY	PAG	PAW	DEG	CAS	ALV	GM	WM
1/SMI-D	+	++++ S>D						
2/SMI		++++ S	++ P					
3/SMI-D		++++ S						
4/SMI	++++ S	++ S						
5/Control	++ S	++++ S						
6/SMI-D		++++ S>D	++ F	+		++ S	+	
7/SMI-D		+++ S	+				++ S	
8/Control		+++ S						
9/SMI-D		+++ S		+			+++ S	
10/SMI[a]		+++ S>D	+				++ S	
11/SMI-D[b]	++ S	+			+	++++ S		
12/SMI-D		+				+++ S		
13/Control		+++ S>D						
14/SMI-D	+	++ S, D, P		+				+++ S
15/SMI-D	+	++ S					+++ S	
16/SMI	++ D, S					+++ S	++ S	
17/Control	+	+++ S					++ S	
18/SMI-D		+++ S						
19/SMI						+++ S		+
20/SMI-D	++++ F	++++ D			+			
21/SMI-D	++ D	++++ D>S			+		++ S	
22/SMI		+++ D			+			
23/Control with MCI	+	+++ D	++ D	+	++ D	+	+	
24/Control with MCI	+++ D	+++ D	+	+++ D	++++ D	++ D	++ D	+
25/SMI-D		+++ F>P						
26/SMI		+		+++ D	++++ D	++ S	+++ S>D	+
27/SMI	+	+	+		++++ D		++ F	+
28/Control			+		+++ D		++ D>S	
29/SMI	+					+	+++ F	+
30/SMI-D		++++ S	++++ P	+		+++ S	+	
31/Control	++ D	+++ D>S	++++ P					
32/SMI-D	+	+	++++ P			+		
33/Control	++++ P							
34/SMI-D		++ S	+++ P				+	
35/Control			+++ P					

(continued)

Table 3. Whole-Brain TDP-43 Pathology in Chronic Psychotic Illness and the Controls 65 Years and Older (continued)

Case No./ Diagnosis	NEO				MES		RHO			DEE	
	TEG	TEW	FRG	FRW	CIG	CWC	MED	CER	MIB	BAS	THA
1/SMI-D											
2/SMI											
3/SMI-D											
4/SMI						++ P, S					
5/Control											
6/SMI-D	++ S				+	+++ S		++ D	+		
7/SMI-D							+++ F>S	++ S			
8/Control											
9/SMI-D											
10/SMI[a]											
11/SMI-D[b]							+++ F		++ F		
12/SMI-D											
13/Control											
14/SMI-D											
15/SMI-D					++ S						
16/SMI											
17/Control										+	
18/SMI-D											
19/SMI	+++ S										
20/SMI-D											
21/SMI-D	+++ D				+					++ P	+
22/SMI											
23/Control with MCI										+	
24/Control with MCI											+
25/SMI-D								+			
26/SMI			+++ S, P								
27/SMI					+				+++ F	++ D	+++ D
28/Control											
29/SMI						++ S				++ S	
30/SMI-D										+	
31/Control											
32/SMI-D									+	+	
33/Control											
34/SMI-D										++++ P	
35/Control						+					

Abbreviations: ALL, allocortex; ALV, alveus; AMY, amygdala; ARC, archicortex; BAS, basal ganglia (striatum/lentiform nucleus); CAS, CA1-CA4–subiculum; CER, cerebellum; CIG, cingulate gray matter; COR, corticoid; CWC, cingulate white matter and corpus callosum; D, diffuse TDP-43 pathology; DEE, deep brain nuclei; DEG, dentate gyrus; F, focal; FRG, frontal gray matter; FRW, frontal white matter; GM, gray matter; MCI, mild cognitive impairment/complaints; MED, medulla; MES, mesocortex; MIB, midbrain; NEO, neocortex; P, perivascular TDP-43 pathology; PAG, periamygdaloid gray matter; PAL, palaeocortex; PAW, periamygdaloid white matter; RHO, rhombencephalon; S, subventricular (subependymal or subpial) TDP-43 pathology; SMI, severe mental illness; SMI-D, severe mental illness with superimposed dementia; TDP-43, 43-kDa transactivation response DNA-binding protein; TEG, temporal gray matter; TEW, temporal white matter; THA, thalamus; TRA, (trans)entorhinal region; WM, white matter; +, rare pathology; ++, mild pathology; +++, moderate pathology; ++++, severe pathology.

[a] Schizoaffective disorder.

[b] Case with variation in progranulin gene.

Diffuse TDP-43 Pathology

Herein, the term *diffuse pathology* refers to a more uniform pathology of varying degree that covers a significant proportion of a given brain area as denoted in Table 3, but it does not imply spread of pathology into wide neocortical brain regions as used elsewhere for pathological TDP-43[31,39] or α-synuclein lesions.[40] Diffuse pathology was found in the CA4-CA1–subiculum area of the hippocampus with predominantly dystrophic neuritic pathology, but also with intermingled neuronal cytoplasmic inclusions (**Figure 3**). In single cases, the absence of microtubule associated protein 2 staining of cellular processes in this brain area was accompanied by TDP-43 dystrophic pathology (data not shown). Diffuse pathology was also present in other brain areas such as the amygdala or, in 1 case (case 21 in Table 3), in the temporal neocortex in a morphological pattern reminiscent of FTLD-U subtype 2, according to Sampathu and colleagues.[41] The diffuse TDP-43 pathology often was found in the same area as tau pathology, however, with only partial colocaliza-

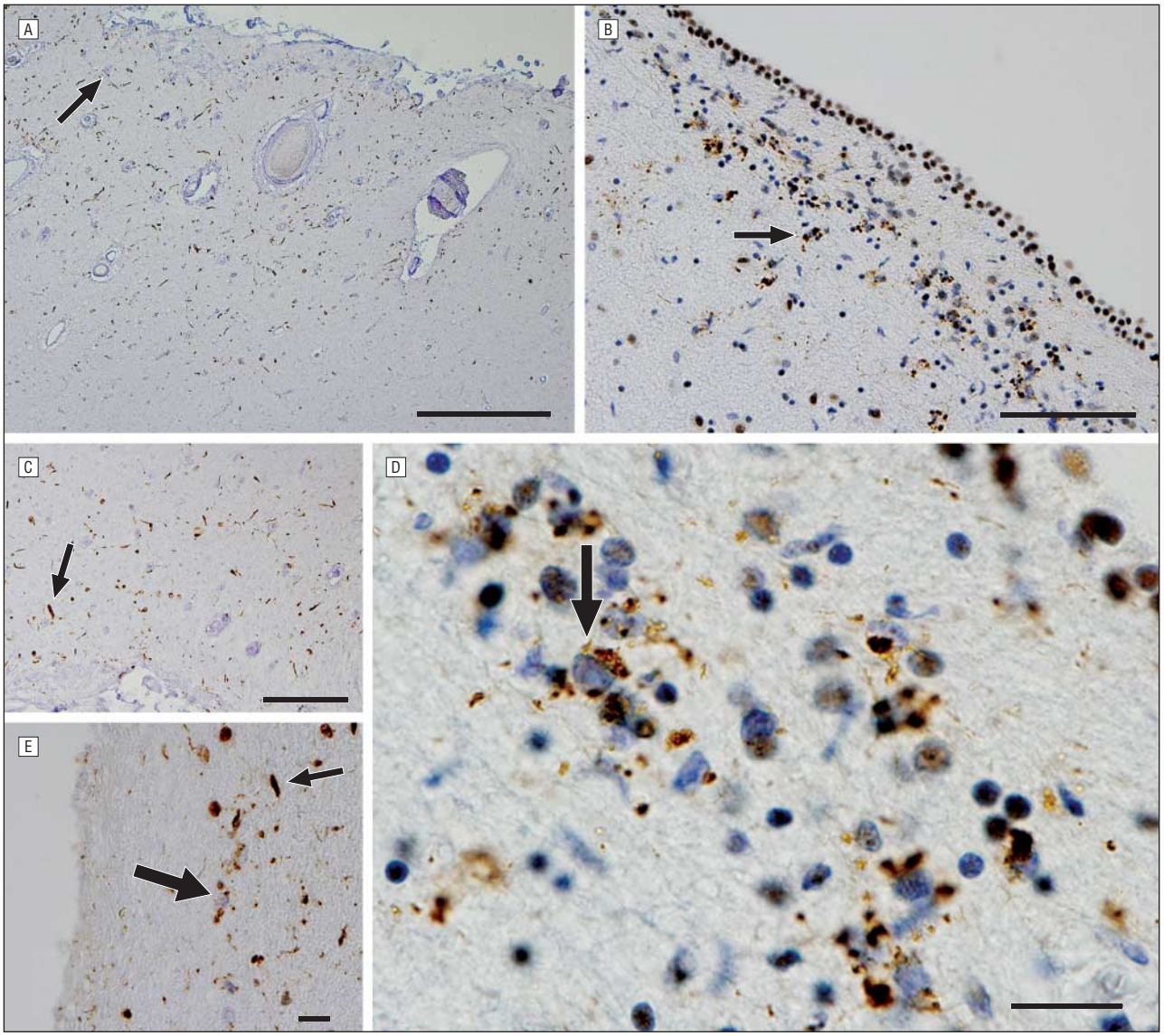

Figure 1. Subpial or subependymal 43-kDa transactivation response DNA-binding protein (TDP-43) pathology. Anti–TDP-43 immunohistochemical examination using phosphorylation-independent (B, D, and E) and antiphosphorylated S409/410 (A and C) TDP-43 antibodies. A, Schizophrenia with superimposed dementia with many subpial dystrophic cellular processes in the periamygdaloid cortex (arrow) (bar=500 µm). B, Amygdala of a subject with schizophrenia showing subependymal cytoplasmic granular TDP-43 immunoreactivity (arrow) (bar=100 µm). C, Higher magnification of part A showing dystrophic cellular processes (eg, arrow) (bar=200 µm). D, High magnification of part B showing granular TDP-43 immunoreactivity (eg, arrow) (bar=20 µm). E, Alveus of a subject with schizophrenia with superimposed dementia showing subependymal cytoplasmic TDP-43 immunoreactivity (large arrow) and dystrophic cellular processes (small arrow) (bar=50 µm).

tion. This variable colocalization was found both in the SMI and control groups and was located in neuronal cell bodies and dystrophic cellular processes including those in neuritic plaques (Figure 3E-J and eFigure 2). Areas with significant TDP-43 showed variable microglia infiltration and astrogliosis consistent with (reactive changes to) a neurodegenerative process (data not shown).

Perivascular TDP-43 Pathology

This pattern of TDP-43 pathology was present predominantly in the form of dystrophic cellular processes or cytoplasmic immunoreactivity in brain areas surrounding medium blood vessels, often focally in the white matter (**Figure 4**). Rarely, pathological TDP-43 associated with small blood vessels/capillaries was present.

GENETIC FINDINGS

DNA sequence analysis of *TARDBP* in 79 patients with schizophrenia revealed no mutations or novel polymorphisms. A heterozygous missense variation of unknown significance, NM_002087.2:c.620T>C, resulting in an amino acid change (p.Met207Thr) was identified in exon 7 of *GRN* in 1 patient with schizophrenia. This novel variant has not been studied previously (see Yu et al[42] for a recent update on currently known *GRN* mutations) but this change is predicted to be benign according to PolyPhen (http://genetics.bwh.harvard.edu/pph/), a Web site that performs automated predictions of the impact an

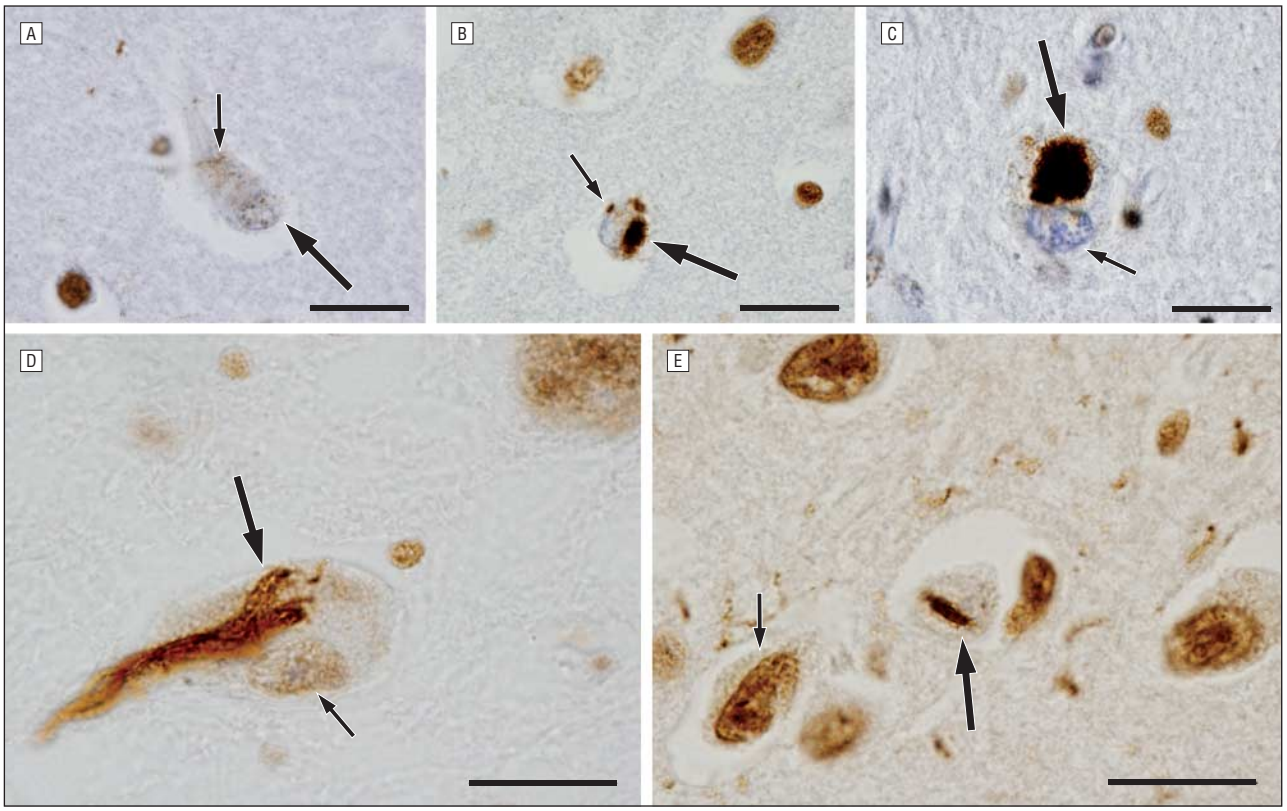

Figure 2. Focal 43-kDa transactivation response DNA-binding protein (TDP-43) pathology. Anti–TDP-43 immunohistochemical examination using phosphorylation-independent (A-F) anti–TDP-43 antibody. A and B, (Trans)entorhinal cortex of a patient with schizophrenia. A, Lack of endogenous TDP-43 staining ("cleared nucleus") (large arrow) and hardly discernable cytoplasmic TDP-43 immunoreactivity (small arrow) (bar=20 μm) in a neuron. B, Medium-size dense neuronal cytoplasmic inclusions (large arrow) and 2 smaller TDP-43 pathological aggregations (small arrow) (bar=20 μm). C, Large neuronal cytoplasmic inclusion (large arrow) and a cleared nucleus (small arrow) subadjacent to the hippocampal pyramidal cell layer in a subject with schizophrenia (bar=20 μm). D, Skeinlike inclusion in a nigral neuron (large arrow) associated with a cleared nucleus in a patient with schizophrenia (small arrow) (bar=20 μm). E, Neuronal intranuclear inclusion (large arrow) in a cell devoid of the endogenous nuclear staining in the hippocampal CA1 area neuron in an elderly control with mild cognitive impairment (bar=20 μm). Note the presence of normal nuclear staining in surrounding neurons (small arrow).

amino acid change will have on the structure and function of a protein.[43]

CLINICOPATHOLOGICAL CORRELATION

Age at death was higher in the group with significant TDP-43 pathology vs the group without (median, 85.0 years [range, 80.0-90.0 years] vs median, 74.5 years [range, 64.0-82.0 years]; $P<.001$). The age at onset of SMI cases was similar (median, 24.0 years [range, 20.2-29.8 years] vs median, 24.0 years [range, 20.0-30.0 years]; $P=.91$). When comparing age at death within either the SMI or the control group, the TDP-43–positive cases had a higher age at death (SMI group: median, 84.0 years [range, 79.5-88.0 years] vs median, 77.5 years [range, 71.8-83.0 years]; $P=.002$; control group: median, 88.5 years [range, 79.2-94.2 years] vs median, 64.0 years [range, 54.8-74.2 years]; $P<.001$). Likewise, the proportion of TDP-43–positive cases within age decades increased with advancing age as shown in **Table 4**. Similarly, the proportions of TDP-43–positive subjects relative to the total number of subjects with significant TDP-43 pathology increased significantly toward higher age category.

Notably, 16 of 25 patients with SMI (64.0%) with significant TDP-43 pathology (either diffuse, focal, or subpial/subependymal pattern) showed a clinical history of deterioration of functional or cognitive abilities (dementia); 2 of the 8 control subjects (20%) with significant TDP-43 pathology exhibited slight cognitive impairment and complaints, respectively (denoted "gray cases" mentioned earlier). Almost all subjects with significant perivascular TDP-43 pathology had recorded clinical or pathological evidence of cardiovascular findings, including hypertension, arteriosclerosis, peripheral vascular disease, or CNS macroinfarcts or microinfarcts.

The patient with schizophrenia with superimposed dementia and the *GRN* gene variant had subependymal TDP-43 pathology in the alveus and amygdala, focal TDP-43 pathology in the parenchyma of the brainstem, and rare TDP-43 pathology in the orbitofrontal cortex. There were no apparent clinical differences in this subject as compared with the other cases in the SMI with dementia group. Since the meaning of the *GRN* variant is unknown and the clinical and pathological evaluation did not reveal any distinguishing features in this patient, the significance of this finding is unclear.

Finally, given the recent finding of FUS pathology in a small subset of FTLD-U cases negative for TDP-43 ("atypical FTLD-U"),[44-46] we evaluated 18 patients with schizophrenia exhibiting deterioration of functional and cognitive abilities (dementia), but without significant TDP-43 pathology, for FUS pathology in the amygdala

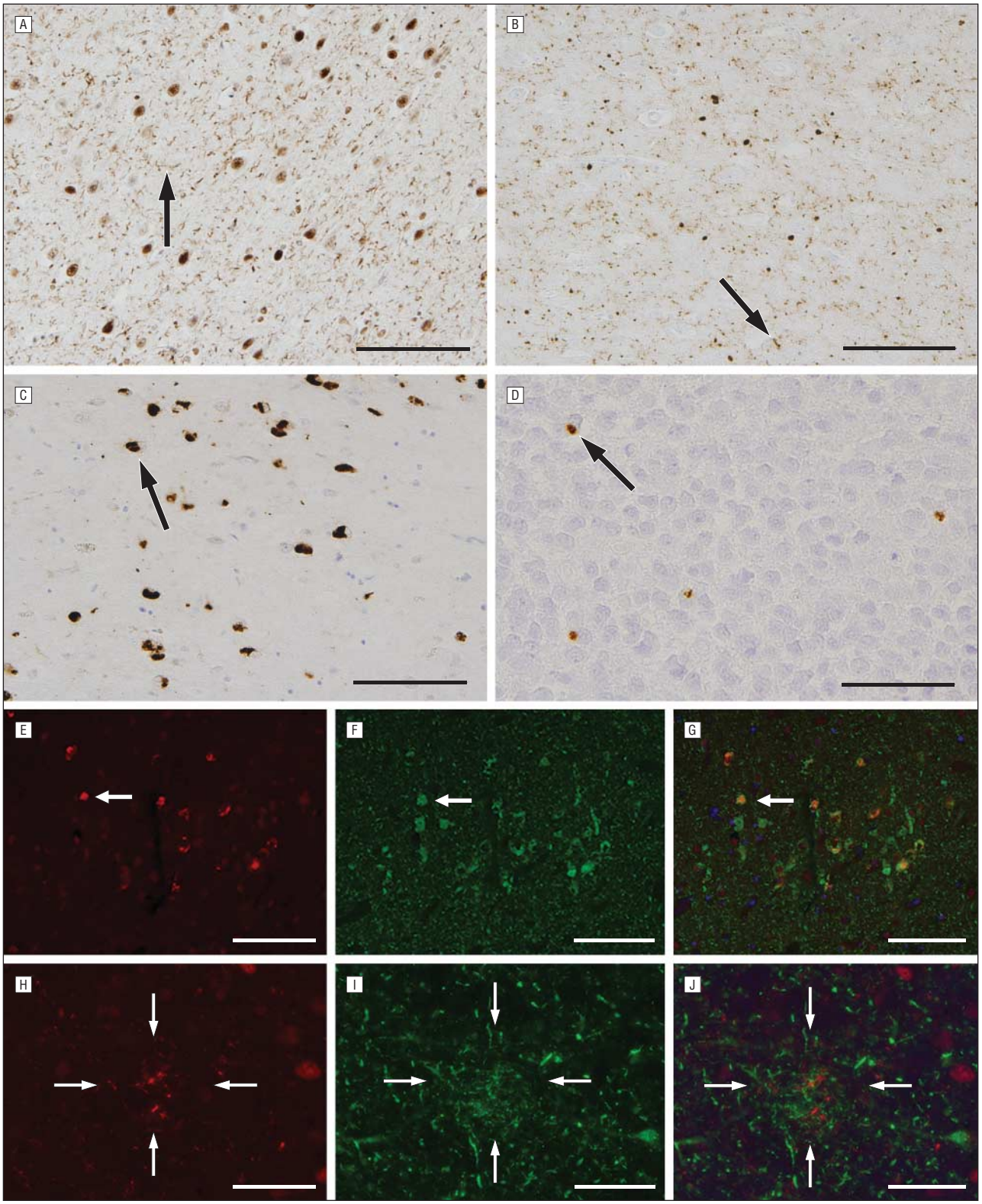

Figure 3. Diffuse 43-kDa transactivation response DNA-binding protein (TDP-43) pathology. Anti–TDP-43 immunohistochemical examination using phosphorylation-independent (A) and antiphosphorylated S409/410 (B-D) TDP-43 antibodies. A, CA1-subiculum area of the hippocampus showing severe dystrophic TDP-43 pathology in a subject with mild cognitive impairment (eg, arrow) (bar=100 µm). B, CA1-subiculum area of the hippocampus showing severe dystrophic TDP-43 pathology in a subject with schizophrenia (eg, arrow) (bar=100 µm). C, Subject with schizophrenia with superimposed dementia with neuronal TDP-43 pathology in the periamygdaloid cortex (bar=100 µm) (eg, arrow). D, Dentate gyrus in the hippocampus of a subject with mild cognitive impairment showing some neuronal cytoplasmic inclusions (eg, arrow). E-J, TDP-43 and tau double immunofluorescence immunohistochemical examination using phosphorylation-independent anti–TDP-43 antibodies (E and H) and paired helical filament-1 antibodies (F and I). E-G, Periamygdaloid cortex of a subject with schizophrenia with superimposed dementia showing variable colocalization of pathological tau (F) and TDP-43 (E) (merge in part G) inclusions (eg, arrow) (bar=100 µm). H-J, Hippocampus of a subject with mild cognitive impairment demonstrating convergence of tau (I) and TDP-43 (H) (merge in part J) pathology in a dystrophic plaque (marked by arrows) (bar=50 µm).

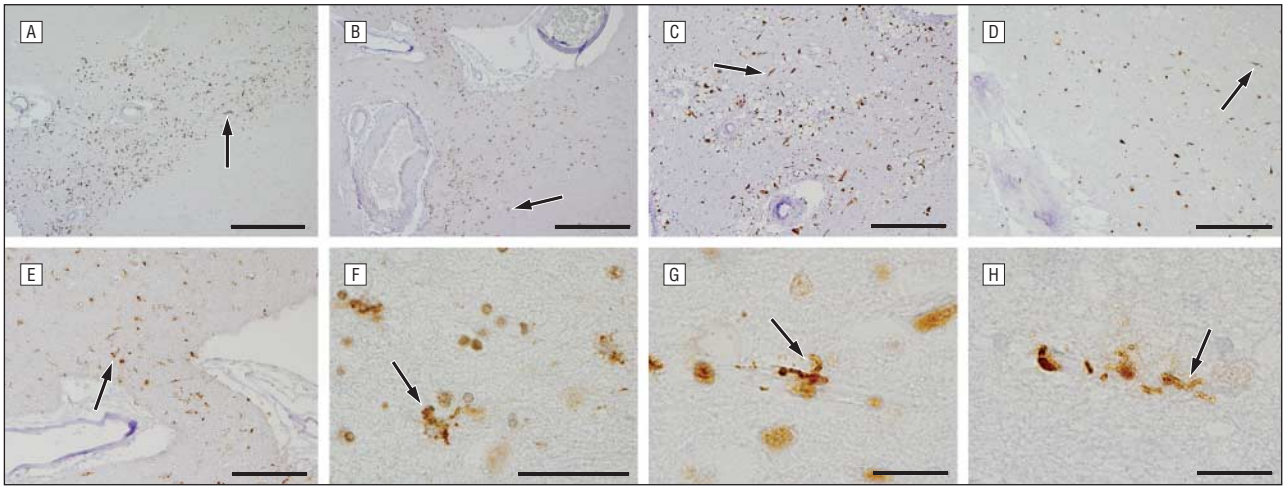

Figure 4. Perivascular 43-kDa transactivation response DNA-binding protein (TDP-43) pathology. Anti–TDP-43 immunohistochemical examination using phosphorylation-independent (F and G) and antiphosphorylated S409/410 (A-E and H) TDP-43 antibodies in the amygdala or periamygdaloid areas. A and B, Low-power magnification of a severe perivascular lesion showing predominantly dystrophic TDP-43 immunoreactive cellular profiles in the periamygdaloid white matter (A) (eg, arrow) or TDP-43 immunoreactivity in the cytoplasm and proximal cellular processes in the amygdala (B) in 2 elderly control subjects (bars=500 μm). C, Same control subject as in part A showing perivascular, mainly dystrophic (eg, arrow), TDP-43 immunoreactivity at a higher magnification (bar=200 μm). D, Subject with schizophrenia with superimposed dementia with perivascular dystrophic TDP-43 pathology in the periamygdaloid white matter (eg, arrow) (bar=200 μm). E and F, Medium-power (E) (bar=200 μm) and high-power (F) (bar=50 μm) views of the same control subject as shown in part B. Note the presence of predominantly cytoplasmic TDP-43 immunoreactivity (eg, arrows), coupled with a lack of endogenous nuclear TDP-43 staining in part F. G and H, Capillary-associated TDP-43 pathology in periamygdaloid gray matter in a patient with schizophrenia and superimposed dementia (arrows) (bars=20 μm).

and/or hippocampus. However, these studies did not show any pathological FUS immunoreactivity as published for atypical FTLD-U.[46]

COMMENT

Ever since the initial definitions of psychotic illnesses were introduced into the literature more than 100 years ago, there has been a debate about a morphological substrate of schizophrenia, and both neurodegenerative and neurodevelopmental pathogenetic theories to explain schizophrenia have been offered. Reports on the frequency or severity of neurodegenerative changes such as AD plaques and neurofibrillary tangles continue with some controversy.[4,6-9] Further, although the significance of pathological TDP-43 for human disease was appreciated only recently, the presence or degree of TDP-43 pathology in controls have not been systematically addressed.[22,30] Also, since the behavioral variant of FTD might overlap clinically with some aspects of schizophrenia, and therefore might be misdiagnosed in life as schizophrenia, we undertook the study described herein to examine the postmortem CNS in a large cohort of patients with chronic SMI, mainly schizophrenia, for the presence of pathological TDP-43, in comparison with control individuals. We found evidence of significant, moderate, or severe pathological TDP-43 in approximately 30% of patients with chronic SMI/schizophrenia and controls 65 years and older, but not in younger individuals; mild pathology was present in approximately another 20% of the elderly study subjects. Moreover, the group with significant TDP-43 pathology was sufficient to create 4 different morphological patterns that sometimes showed overlap in a given case.

A significant proportion of cases showed TDP-43 pathology comprising dystrophic cellular processes and preinclusions located closely subjacent to ventricular surfaces with a focal or bandlike appearance. The significance of this finding is unknown, and further studies are needed to define the relationship of TDP-43 lesions with brain surfaces because this might imply an association between mechanisms of TDP-43 with processes involving the ependymal lining and the cerebrospinal fluid system. Mechanistic speculations might thus include a bidirectional interaction of the CNS and the cerebrospinal fluid, and it is known that there is an increase in cerebrospinal fluid TDP-43 levels in patients with FTLD-U and amyotrophic lateral sclerosis compared with controls.[47,48]

A further morphological pattern comprised single foci of pathology either in the gray matter (often neuronal cells with a cleared nucleus coupled with stained dystrophic cellular processes) or in the white matter (mainly dystrophic cellular processes). Focal gray matter lesions could represent an early stage of TDP-43 pathology formation similar to what has been described for neurofibrillary tangle pathology in the (trans)entorhinal cortex.[49] We herein show that patients with chronic SMI and controls show pathological TDP-43 aggregates in different stages of their development ranging from early, more diffuse punctuate immunoreactivity to "mature," larger and denser inclusions including TDP-43 immunoreactivity in cellular processes. Very upstream stages of inclusion formation may include almost no discernable cytoplasmic pathological TDP-43 aggregates combined with an absence of normal nuclear immunoreactivity ("cleared nuclei"), but it remains to be established if a nucleus devoid of normal TDP-43 without cytoplasmic TDP-43 immunoreactivity might represent incipient TDP-43 pathology. Notably, changes such as this were present in all cohorts including subjects with schizophrenia with and without dementia and controls with and without mild cognitive "issues," so this might support

Table 4. Age-Related Frequency of Significant TDP-43 Pathology in Patients With Chronic Severe Mental Illness and Control Subjects

Age Category, y	Significant TDP-43 Pathology in the Hippocampus/(Trans)entorhinal Cortex and/or Amygdala/Periamygdaloid Region		
	No. of TDP-43–Positive Cases (n=35)	% of Age Category[a]	% of Total Positive Cases[a]
<64 (n=31)	0	0	0
65-74 (n=31)	4	12.9	11.4
75-84 (n=49)	12	24.5	34.3
≥85 (n=40)	19	47.5	54.3
P value[a]		<.001	<.001

Abbreviation: TDP-43, 43-kDa transactivation response DNA-binding protein.
[a] χ^2 Test.

the idea of very early cellular pathology with the beginning of TDP-43 redistribution into the cytoplasm not severe enough yet to cause significant neuronal loss and reactive changes.

Diffuse TDP-43 pathology was present in the CA1-subiculum area of the hippocampus mainly consisting of abundant short dystrophic cellular processes intermingled with preinclusions or more "mature," dense inclusions. This was sometimes in the same brain areas as neurofibrillary and neuritic tau pathology, but colocalization of tau and TDP-43 pathology was variable. To our knowledge, we are the first to show that this partial colocalization of tau and TDP-43 pathology does also occur in neuritic plaques. TDP-43 pathology is known to occur in patients with AD and hippocampal sclerosis and this includes abundant dystrophic TDP-43 pathology in the subiculum-CA1 region of the hippocampus.[31,35,39,50] Besides the CA1-subiculum pathology, diffuse pathology was found in the amygdala or periamygdaloid cortex and, less frequently, in other cortical areas. The colocalization with tau lesions was also variable. This pattern observed herein is reminiscent of the co-occurrence of TDP-43 pathology in pathologically diagnosed cases of AD.[51] Also, in addition to the subiculum-CA1 involvement, TDP-43 pathology has also been reported in the parahippocampal gyrus and entorhinal cortex, whereas the dentate gyrus was affected variably or rarely.[50,51] In the present study, cytoplasmic inclusions in the dentate nucleus were uncommon both in the SMI/schizophrenia and control groups similar to a previous report showing infrequent inclusion in the fascia dentata of Guamanian controls.[21] This TDP-43 finding is paralleled by published data on robust tau-positive inclusion in the fascia dentata occurring in advanced stages of AD rather than in low-grade cases.[49]

It was previously suggested that in advanced AD medial temporal lobe limbic structures are vulnerable to TDP-43 pathology, with the amygdala being the most susceptible region, implying a progression of TDP-43 pathology with higher-order association cortices being affected only later on (or in a subset of cases) and other limbic brain areas having an intermediate position.[39,51] Neocortical pathology was present only exceptionally in our cohort. The fact that about a quarter of subjects with diffuse pathology in the hippocampus or amygdala also exhibited TDP-43 lesions in other areas, such as the rhombencephalon, deep brain nuclei, or neocortex, corroborates the multisystem concept of TDP-43 proteinopathies.[13]

In a subset of controls and subjects with schizophrenia, there appeared to be a greater abundance of TDP-43 pathology around blood vessels, ie, in the perivascular white matter or, less frequent, gray matter, but further studies are needed to confirm and extend this association. Moreover, most of these cases had a documented clinical history of cardiovascular problems or pathology related thereto. Considering the high prevalence of chronic vascular changes in elderly individuals, there might not necessarily be a link between ischemia and pathological TDP-43,[52] but the findings herein suggest further study of this possibility. Although it also remains to be established what the localization of TDP-43 pathology around blood vessels means in terms of a hypothesized "interaction" between blood and the brain, it has been suggested recently that increased TDP-43 plasma levels occur in FTLD-U and AD and may thereby index TDP-43 pathology within the brain.[53] Interestingly, another recent article reported on a "TDP-43 microvasculopathy" in FTLD-U (and familial Lewy body disease) and suggested that abnormal TDP-43 fibrillary inclusions may occur in astrocytic end-feet, raising the possibility of an impairment in the integrity of the blood-brain barrier.[54]

The finding of a higher age at death in the TDP-43 pathology–positive as compared with the TDP-43 pathology–negative group and, similarly, the steady increase of the frequency of pathological TDP-43 per age decade after the age of 65 years, denotes an aging-related deposition of this pathological protein with the pathology burden being higher in older subjects. This is paralleled by varying degrees of pathology of many disease proteins including tau-, β-amyloid–, and α-synuclein–related pathological aggregates in the CNS of an elderly, neurologic and cognitive normal or only mildly impaired population.[15,17] The recent report on an absence of TDP-43 pathology in 8 patients with schizophrenia (or a schizoaffective disorder) might be, at least in part, due to their relatively young age with death occurring in their early 60s.[18]

The term *atypical FTLD-U* was recently coined referring to sporadic early-onset FTD with severe progressive behavioral and personality changes in the absence of aphasia or significant motor features[44,45] and was, most recently, associated with FUS inclusion pathology.[46] De-

spite some clinical similarities between schizophrenia with superimposed deterioration in functional and cognitive abilities (dementia) and atypical FTLD-U, the TDP-43 pathology–negative schizophrenia cohort with the clinical presence of dementia did not show any atypical FTLD-U–like FUS pathology, implying different disease mechanisms between these 2 disorders. However, the early nonmemory symptoms in FTD, such as addictive behaviors or disinhibition,[55] resembling some aspects of schizophrenia could be due to the TDP-43 pathology in limbic brain areas as shown in a subset of schizophrenia cases in this study.

CONCLUSIONS

We herein show that pathological TDP-43 is present in a subgroup of patients with chronic SMI, mainly schizophrenia, and controls, with the burden of TDP-43 pathology being higher with advancing age. While TDP-43 pathology might underlie behavioral impairments or dementia in these patients with SMI/schizophrenia, the presence of similar pathology in controls suggests that the findings may be related to aging. Although no diagnostic CNS topographical algorithms for the emergence of TDP-43 pathology have been developed yet, these results may signify that limbic regions are the earliest affected in the course of a cerebral TDP-43 multisystem proteinopathy. However, this view requires further study and the data presented provide an age-related baseline for the prevalence of TDP-43 in controls, which will be important for developing whole-brain evolution schemes for TDP-43 pathology in neurodegenerative TDP-43 proteinopathies.

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