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Hippocampal sclerosis in Lewy body disease is a TDP-43 proteinopathy similar to FTLD-TDP Type A

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Abstract

Hippocampal sclerosis (HpScl) is frequent in frontotemporal lobar degeneration with TDP-43 pathology (FTLD-TDP), but it also occurs in dementia of the elderly with or without accompanying Alzheimer type pathology. HpScl has been hypothesized to be a neurodegenerative process given its association with TDP-43 pathology, but this is still controversial. TDP-43 pathology is found in Lewy body disease (LBD), but no study has focused on the pathologic and genetic characteristics of HpScl in LBD. We found HpScl in 5.2% of 669 LBD cases (289 transitional and 380 diffuse). Older age, higher Braak neurofibrillary tangle (NFT) stage, and presence of TDP-43 pathology were associated with HpScl. There was no difference in the frequency of HpScl between transitional and diffuse LBD, suggesting that Lewy related pathology appears to have no direct association with HpScl. All HpScl cases had TDP-43 pathology consistent with Type A pattern. HpScl cases harbored genetic variation in *TMEM106B* that has been previously associated with FTLD-TDP. Interestingly, the severity of TDP-43-positive fine neurites in CA1 sector, a possible pathologic precursor of HpScl, was associated with the *TMEM106B* variant. These results demonstrate HpScl in LBD is a TDP-43 proteinopathy and is similar to FTLD-TDP Type A. Furthermore, a subset of LBD cases without HpScl (“pre-HpScl”) had similar pathologic and genetic characteristics to typical HpScl, suggesting that the spectrum of HpScl pathology may be wider than previously thought. Some cases with many extracellular NFTs also had a similar profile. We suggest that HpScl is “masked” in these cases.

Keywords

Hippocampal sclerosis; Lewy body disease; Neuropathology; TDP-43; *TMEM106B*

INTRODUCTION

Hippocampal sclerosis (HpScl) in the elderly is a pathologic diagnosis characterized by selective neuronal loss and gliosis in CA1 sector and/or subiculum of the hippocampus [6,24]. The clinical manifestation is slowly progressive amnesic dysfunction. In many patients with dementia, HpScl coexists with other neurodegenerative disorders [31], including Alzheimer's disease (AD), frontotemporal lobar degeneration with TDP-43 pathology (FTLD-TDP), and Lewy body disease (LBD). The prevalence of HpScl in autopsy series of dementia ranges from 2.8% to 23.4% [31]. It is most frequently associated with FTLD-TDP and can be seen in approximately 70% of FTLD-TDP cases [11]. It is also relatively common in AD cases, and the reported prevalence is about 10%. Occasionally, it has been reported as the only pathologic explanation for dementia and is termed "pure-HpScl" [1]. The prevalence of "pure-HpScl" ranges from 0.4% to 4.6%.

The etiology of HpScl in the elderly is still unknown, but it has been hypothesized to be a neurodegenerative process given its association with TDP-43 pathology [2]. TDP-43 pathology was initially considered to be a specific marker for FTLD-TDP [26]; however, it is increasingly clear that TDP-43 pathology is also seen in some AD cases and in most AD cases with concurrent HpScl [2,5,21,24,27]. TDP-43 pathology was detected in 19% to 57% of AD cases [2,12] and about 80% of AD with HpScl cases [5]. While hypoxic damage causes a similar distribution of neuronal loss in the hippocampus, TDP-43 pathology is not detected in such cases. Recent studies have also shown genetic similarity between HpScl and FTLD-TDP. One of the most common genetic causes of FTLD-TDP is a mutation in the progranulin (*GRN*) gene, and most of these mutation carriers have HpScl [10,16,17]. In addition to causative mutations, two genetic risk variants have been identified as common risk factors for FTLD-TDP and HpScl in AD [5,21,29]. One is located in the 3' untranslated region of *GRN* (rs5848), and the other is located in the uncharacterized transmembrane protein *TMEM106B* (rs1990622).

Lewy body disease (LBD) is pathologically characterized by Lewy bodies (LBs) and Lewy neurites, which are composed of α -synuclein [13,19]. LBD is accompanied by varying degrees of Alzheimer type pathology, including neurofibrillary tangles (NFTs) and senile plaques (SPs). LBD cases in this study had a range of clinical presentations including Parkinson's disease, Parkinson's disease with dementia, dementia with Lewy bodies (DLB), and other syndromes [14]. Recent studies have demonstrated that TDP-43 pathology is relatively common in LBD, with the reported frequency ranging from 18% to 60% [3,9,22,30]. The presence of TDP-43 pathology in LBD is associated with severity of tau pathology; however, the influence of α -synuclein pathology on the accumulation of TDP-43 pathology is poorly understood. As far as we know, there are no reports on the frequency and characteristics of HpScl in LBD.

The pathologic diagnosis of HpScl in the elderly can be difficult if abundant AD pathology coexists because NFTs also cause neuronal loss in similar regions of the hippocampus. In such cases, the diagnosis of HpScl is warranted only when neuronal loss is disproportionate to the number of extracellular NFTs. Given the high frequency of TDP-43 pathology in HpScl [2,24,25,27], TDP-43 immunohistochemistry can be helpful to the diagnosis of

HpScl. Hatanpaa et al. reported [8] that TDP-43-positive fine neurites in the pyramidal layer of the hippocampus might be a precursor lesion of the HpScl in FTLN-TDP. Whether this also applies to TDP-43 pathology in HpScl in the elderly is not fully understood.

The aim of this study was to investigate pathologic and genetic characteristics of HpScl in LBD. In addition, we examined whether the severity and subtype of TDP-43 pathology differed between LBD cases with and without HpScl. The findings presented in this report contribute to increasing evidence that HpScl in the elderly might share disease processes with FTLN-TDP. Furthermore, results of immunohistochemical analysis of TDP-43 pathology indicate that the pathologic spectrum of HpScl might be wider than previously considered.

MATERIALS AND METHODS

Case material

The database of the brain bank at Mayo Clinic in Jacksonville was queried for consecutive cases received between 1997 to 2013 with a neuropathologic diagnosis of either limbic or diffuse LBD [13] in which slides were available for review and paraffin blocks were available for further research. There were 669 LBD cases: 380 diffuse and 289 transitional LBD. We excluded cases of incidental LBD, brainstem predominant LBD, amygdala predominant Lewy bodies in AD, and LBD cases harboring known pathogenic mutations. LBD found concurrent with other major neurodegenerative disorders, such as FTLN-TDP, progressive supranuclear palsy, corticobasal degeneration, Pick's disease, and multiple system atrophy were excluded. 82% (547/669) of cases were classified as high- or intermediate-likelihood DLB based on the Third Consortium on DLB pathologic criteria [19]. Of the 669 LBD cases in our series, 248 (37%) overlapped with a recently published study investigating HpScl in the setting of advanced AD [21].

Neuropathologic methods

All cases had undergone neuropathologic assessment by a single neuropathologist (DWD), which included standardized tissue sampling and quantitative assessment of AD and Lewy pathology. Thioflavin-S fluorescent microscopy was used for the evaluation of SP and NFT densities in multiple brain regions, including 6 cortical sections, 4 sectors of the hippocampus, and 2 regions of the amygdala. NFTs were also assessed in the basal nucleus of Meynert and presence of SP was assessed in basal ganglia and cerebellum. The distribution of NFTs was used to assign a Braak NFT stage [4] and the distribution of SPs was used to assign a Thal amyloid- β (A β) phase, as previously described [20]. Immunohistochemistry was performed on all cases with α -synuclein antibody (NACP, 1:3000 rabbit polyclonal, Mayo Clinic antibody), and the LB densities in 5 cortical sections and the amygdala were assessed. The distribution of LBs was used to assign the subtypes of Lewy body pathology. In addition, immunohistochemistry with phosphorylated tau antibody (PHF-1, 1:1000 mouse monoclonal, gift from Dr. Peter Davies) was also performed in a subset of cases.

Diagnosis of HpScl

HpScl was assessed on hematoxylin-eosin stained sections. It was diagnosed when neuronal loss and gliosis was selective in the CA1 sector and/or subiculum of the hippocampus in the absence of other pathologic findings that could account for neuronal loss in this region. In cases with NFTs, neuronal loss had to be disproportionate to the density of extracellular NFTs.

TDP-43 immunohistochemistry

A 5-um-thick paraffin section of the hippocampus was screened with TDP-43 immunohistochemistry (MC2085 [32], 1:2500 rabbit polyclonal, from Leonard Petrucelli, Mayo Clinic, or with 10782-2-AP, 1:3000 rabbit polyclonal, Protein Tech Group, Chicago) in all cases using a DAKO Autostainer (Universal Staining System Carpinteria, California). In this study, qualitative and quantitative TDP-43 pathology was analyzed on sections that were immunostained with phosphorylated TDP-43 antibody (pS409/410, 1:5000 mouse monoclonal, Cosmo Bio Co., LTD).

All cases were subclassified based on the morphology and distribution of TDP-43 pathology in the hippocampus and adjacent cortex. In addition, a subset of the cases was studied with double-labeling immunohistochemistry with the combination of TDP-43 antibody (MC2085) and phosphorylated tau antibody (PHF-1) using previously published methods [15].

Criteria for subclassification

The Mackenzie scheme [18] was used to classify the cases with modification because it was established for FTLTDP and did not provide a means for classifying TDP-43 that occurs in the setting of AD and LBD. Type A is characterized by numerous neuronal cytoplasmic inclusions (NCIs) and dystrophic neurites (DNs) and occasional neuronal intranuclear inclusions (NIIs). Type B is characterized by numerous NCIs with minimal DNs and no NIIs. Type C is characterized by abundant DNs with minimal NCIs and no NIIs.

Of the 168 TDP-43-positive cases 101 were Type A and 43 cases had a pattern similar to Type B, but they also had variable numbers of NFTs with TDP-43 immunoreactivity. This NFT-like TDP-43 pathology was initially described in AD cases [2], but was not considered in FTLTDP scheme. We refer to this pattern of TDP-43 pathology as “Type B/NFT” in this study. The remaining 24 cases could not be assigned due to sparse TDP-43 pathology in the hippocampus and adjacent cortex. No case was assigned to Type C.

Semi-quantitative assessment of TDP-43 pathology

Semi-quantitative assessment of TDP-43 pathology was performed with pS409/410 antibody for all 35 HpScl cases and 32 randomly selected LBD cases without HpScl. We examined the presence and severity of TDP-43 pathology in the hippocampus, parahippocampal gyrus, inferior temporal cortex, mid-frontal cortex, amygdala, putamen, substantia nigra, and hypoglossal nucleus. Cytoplasmic lesions and neuritic lesions were scored separately using a 5 point grading scale (0= none, 0.5= rare, 1= mild, 2=moderate, 3=severe) by a single neuropathologist (N.A.) who was blinded to genetic data. Cytoplasmic lesions included

NCIs and NFT-like TDP-43 pathology. Neuritic lesions included DNIs and fine neurites in the pyramidal layer of the hippocampus as described by Hatanpaa [8]. The presence of NIIs was also assessed. In addition, the severity of fine neurites in the CA1 sector was assessed in all 101 Type A cases.

Genetic methods

For genotyping, genomic DNA was extracted from frozen brain tissue by standard procedures. Genotyping for *GRN* (SNP rs5848 C/T), *TMEM106B* (SNP rs1990622 T/C), and *APOE* alleles (SNP rs429358 T/C and rs7412 C/T) were assessed with Taqman SNP genotyping assays (Applied Biosystems, Carlsbad, CA) as previously reported [28,29]. Genotype calls were obtained with SDS v2.2.2 software (Applied Biosystems).

Statistical methods

SigmaPlot 12.0 (San Jose, CA) was used to analyze all statistical data and create graphs. A Kruskal-Wallis one-way analysis of variance on ranks or Mann-Whitney rank sum test was performed for group comparisons of continuous variables, as appropriate, and a post-hoc pairwise comparison was performed with Mann-Whitney rank sum test. A Chi-square test was performed for group comparisons of categorical data.

RESULTS

A summary of the demographic and neuropathologic characteristics of all LBD cases in this study is shown in Table 1. HpScl was detected in 35 of 669 cases (5.2%). The HpScl group had a significantly older median age at death than the Not HpScl group (81 vs. 78 years, $P=0.010$). In the HpScl group, 89% of cases were ≥ 75 years of age, while 68% of cases in the Not HpScl group were aged ≥ 75 years. There was no significant difference in the ratio of gender between the two groups ($P=0.197$).

AD and Lewy Pathology

The median brain weight was significantly less in the HpScl group than in the Not HpScl group (1000 vs. 1140g, $P<0.001$). The median Braak NFT stage and Thal A β phase were significantly greater in HpScl group than the Not HpScl group (NFT stage; VI vs. IV, $P<0.001$, A β phase; 5 vs. 4, $P=0.003$). The prevalence of HpScl was significantly higher in the cases with more advanced Braak NFT stages (Table 2) and Thal A β phases. HpScl was detected in 9.1% of the cases with Braak NFT stages V–VI, while HpScl was detected in only 2.8% of those in stages 0–II. Similarly, HpScl was detected in 7.7% of the cases with a Thal A β phase of 4–5, while it was only detected in 2.7% of phase 0–1 cases. In contrast, the prevalence of HpScl was not associated with subtype of Lewy pathology (Table 1 and 2). The ratio of diffuse LBD was similar in the HpScl group and Not HpScl group (60% and 57%, $P=0.828$) (Table 1). HpScl was shown in 5.5% of diffuse LBD cases and 4.8% of limbic LBD cases ($p=0.828$) (Table 2).

The associations between HpScl, Braak NFT stage, Thal A β phase, and age at death were assessed by a multiple logistic regression model. Braak NFT stage and age at death were independently associated with HpScl (Braak NFT stage: OR 1.6, 95% confidence interval

1.08–2.32; age at death: OR 1.05, 95% confidence interval 1.004–1.100). In contrast, association of A β phase with HpScl was not significant in the multiple logistic model ($P=0.940$).

TDP-43 pathology

TDP-43 pathology was found in all of the HpScl cases (35/35), while it was found in only 21% of the cases without HpScl (133/634) ($P<0.001$) (Table 1). Of note, all HpScl cases exhibited Type A morphology and distribution. In contrast, the subtype of the Not HpScl group was heterogeneous. Type A was the most frequent subtype (50%), followed by Type B/NFT (32%). A subset (18%) of cases with TDP-43 pathology could not be classified because the density of lesions was too sparse. No cases of Type C were observed.

The overall prevalence of TDP-43 pathology was 25% (168/669). As previously reported [22], the presence of TDP-43 pathology was associated with higher Braak NFT stage (Table 2). For example, TDP-43 pathology was detected in 40% of cases with Braak stages V–VI, while it was detected in only 7.8% of cases with Braak stages 0–II. There was a trend for TDP-43 pathology to be more frequently in diffuse LBD than limbic LBD, but this was not significant (28% vs. 22%, $P=0.102$) (Table 2).

Given that a subset of the LBD cases with advanced AD pathology were included in a previous report [21], we analyzed characteristics of only the LBD cases not included in the previous series (Supplemental Table 1). When cases that overlapped were excluded, the demographic characteristics were similar, but Alzheimer type pathology measures (i.e., Braak NFT stage and Thal amyloid- β phase) differed since LBD cases with minimal AD pathology were not included in the previous report. In both series HpScl cases had TDP-43 pathology consistent with Type A pattern.

Semi-quantitative assessment of TDP-43 pathology

To clarify the characteristics of TDP-43 pathology in HpScl cases, we performed a semi-quantitative assessment of TDP-43 pathology using all 35 HpScl cases. In addition, we examined 32 randomly selected LBD cases without HpScl (Type A; 19 cases, Type B/NFT; 13 cases) for comparison purposes (Table 3).

HpScl-Type A—All cases had variable density of NCIs in the dentate fascia (Fig. 1a). Fine neurites in CA1 (Fig. 1b and c) and subiculum (Fig. 1d), which have been suggested to be a pathologic precursor for HpScl in FTLD-TDP [8], were found in all cases, with significantly more in CA1 ($P=0.027$). In comparison, NCIs were sparse in CA1. All cases had a mixture of NCIs and short DNs in the parahippocampal gyrus (Fig. 1e). In the inferior temporal gyrus, 74% of the cases had similar pathology as the parahippocampal gyrus, but to a lesser extent (Fig. 1f). The amygdala was also affected in all cases; variable shapes of NCIs and short DNs were found (Fig. 1g). Extension of the pathology beyond the limbic and inferomedial temporal lobe was present in about 30% of the cases, but lesion density in these areas was sparse. TDP-43 pathology was found in 17% and 27% of the cases in the mid-frontal gyrus and putamen (Fig. 1h), respectively. In the substantia nigra (SN), sparse DNs and NCIs were found in 44% and 12% of the cases, respectively (Fig. 1i). No cases had

TDP-43 pathology in the hypoglossal nucleus. NIIs were found in 89% of the cases, most frequently in the dentate fascia, CA1, and parahippocampal gyrus. In summary, the characteristic features of TDP-43 pathology in LBD cases with HpScl was consistent with FTLD-TDP Type A; however, the pathology was relatively restricted to limbic and inferomedial temporal lobe in about 70% of the cases.

Not HpScl-Type A—In general, TDP-43 pathology was relatively mild in this group compared with HpScl-Type A. Significant differences were found in the dentate fascia and putamen, as well as in the presence of NIIs. There was a trend for the density of CA1 fine neurites to be less than in HpScl-Type A ($P=0.097$); however we also found that 42% of the cases in this group had moderate-to-severe CA1 fine neurites similar to HpScl cases. These findings suggest that a subset of the cases in the Not HpScl-Type A group had similar characteristics and severity of TDP-43 pathology to HpScl cases and may represent “pre-HpScl.”

Not HpScl-Type B/NFT—This group was clearly distinguished from HpScl-Type A due to sparse or no neuritic lesions in the hippocampus (Fig. 2a–c), parahippocampal gyrus (Fig. 2d), and inferior temporal gyrus. No cases had TDP-43 fine neurites in CA1 (Fig. 2b) or subiculum (Fig. 2c). In contrast, NCIs were frequent. The dentate fascia had NCIs in 70% of the cases, although they were sparse (Fig. 2a). All cases had NFT-like TDP-43 pathology in the subiculum with sparse or no NCIs (Fig. 2c). A mixture of NCIs and NFT-like TDP-43 pathology was found in the parahippocampal gyrus (Fig. 2d) and amygdala of all cases. The amygdala was the most severely affected region in this group. No case had TDP-43 pathology beyond the limbic and inferomedial temporal lobe. Double-labeling immunohistochemistry demonstrated that the NFT-like TDP43 pathology co-localized with tau pathology.

Genetic findings

We performed genetic analyses to ascertain whether the reported genetic risk variants for FTLD-TDP and HpScl in AD could also contribute to the development of HpScl in LBD. The *TMEM106B* rs1990622 variant, where the C allele correlates with an increase in GRN expression [7], is reported to be protective for FTLD-TDP and HpScl in AD [21,29]. The *TMEM106B* C allele was found significantly less often in the HpScl group than in the Not HpScl group (44% vs. 70%, $P=0.003$) (Table 1). 9.4% of the cases in the HpScl group were homozygous for the protective C allele; this was less than the Not HpScl group (23%). In comparison, the *GRN* rs5848 variant, where the T allele is associated with lower GRN expression, is reported to be a risk factor FTLD-TDP and HpScl in AD [5,21]. The frequency of the cases having the *GRN* T allele in the HpScl group was also higher than the Not HpScl group, but this was not statistically significant (66% vs. 53%, $P=0.209$) (Table 1). In addition, we also examined *APOE* $\epsilon 4$ allele association with HpScl, but found no statistically significant differences between HpScl and Not HpScl groups (66% vs. 54%, $P=0.293$).

Excluding LBD cases that were included in a previous study of advanced AD [21] gave similar genetic findings (Supplemental Table 1) as in the entire LBD cohort. The frequency

of the *TMEM106B* C allele in HpScl was lower than in Not HpScl (50% vs. 67%), although given the smaller sample size this did not reach statistical significance ($P=0.288$). The frequency of the cases having protective *TMEM106B* CC genotype was 7.1% and 22% in HpScl compared to Not HpScl, respectively.

A comparison of demographics and genetic characteristics by subtype of TDP-43 pathology is shown in Table 4. There were no differences in gender and age at death between the HpScl-Type A, Not HpScl-Type A, or Not HpScl-Type B/NFT groups. In contrast, genetic characteristics were different between the HpScl-Type A and the other groups. The *TMEM106B* C allele was found significantly less often in HpScl-Type A than in Not HpScl-Type A (44% vs. 76% $P=0.005$) and Not HpScl-Type B/NFT (44% vs. 74% $P=0.027$). The frequency of the cases having the *GRN* T allele in HpScl-Type A was also higher than in the others, but this was not statistically significant. The difference in the frequency of *APOE* $\epsilon 4$ allele was also not statistically significant between HpScl-Type A and the others.

The genetic findings suggest that the *TMEM106B* variant strongly influences the development of HpScl in LBD, as was observed for HpScl in AD, while the influence of the *GRN* variant was weaker.

CA1 fine neurites and *TMEM106B* genotype

Given the pathologic findings that TDP-43-positive fine neurites might be pathologic precursor of HpScl, we hypothesized that (1) the severity of this pathology might be associated with the *TMEM106B* genotype in Type A cases and (2) Not HpScl-Type A cases with abundant CA1 fine neurites might have similar genetic characteristics as HpScl-Type A cases. To address this issue, we examined all Type A cases that we had access to, including 32 HpScl-Type A cases (Fig. 3a) and 55 Not HpScl-Type A cases (Fig. 3b and c). In addition, we divided the Not HpScl-Type A group into a High CA1 fine neurites group (Fig. 3b) and a Low CA1 fine neurites group (Fig. 3c). Cases with High fine CA1 neurites ($n=30$) had semiquantitative scores of 2 to 3, and cases with Low CA1 fine neurites ($n=25$) had scores 0 to 1. Interestingly, the severity of CA1 fine neurites was significantly less in Type A cases having the protective CC genotype than in the cases having the TT genotype ($p=0.002$) and CT genotype ($P=0.018$) (Fig. 3d). Furthermore, Not HpScl-Type A cases with High fine CA1 neurites also had decreased frequency of the *TMEM106B* CC genotype (10%), which is similar to HpScl-Type A cases (9.4%) (Fig. 3e). These findings suggest that a subset of the LBD cases in the Not HpScl-Type A group had similar pathologic and genetic characteristics to HpScl cases. We considered these cases to be on the spectrum of HpScl pathology; however, they were not diagnosed as HpScl due to the absence of neuronal loss or the presence of many extracellular NFTs in the CA1 sector. We classified these cases as “pre-HpScl” if there was none to minimal neuronal loss or “masked HpScl” if there was neuronal loss, but many extracellular NFTs (Fig. 4).

To determine if the spectrum of HpScl pathology also applies to non-LBD cases, we performed a validation study using 132 non-LBD cases without HpScl or advanced AD pathology (Braak NFT stage III) by TDP-43 immunohistochemistry. The non-LBD validation cohort is described in Supplemental Methods. We found a single case that fit with “pre-HpScl” that had many TDP-43-positive fine neurites and absence of neuronal loss or

extracellular NFTs in the CA1 sector (Supplemental Figure). This 86 year-old man pathologically had vascular ischemic dementia based on presence of multiple lacunar infarcts in the basal ganglia, thalamus, and cerebral white matter. TDP-43 pathology was consistent with Type A, and it was restricted to limbic and inferomedial temporal lobe. Braak NFT stage was II, Thal amyloid- β phase was 4, and there was no argyrophilic grain disease. There was no significant difference in the frequency of “pre-HpScl” between the validation cohort and LBD cohort with Braak NFT stage 0-III (0.76% vs. 2.7% $P=0.354$) (Supplemental Table 2).

DISCUSSION

In this study, HpScl was detected in 5.2% of the LBD cases. We demonstrated that HpScl in LBD is a TDP-43 proteinopathy associated with older age and greater AD pathology, but not with distribution of LBs. TDP-43 pathology was detected in all HpScl cases and 21% of the cases without HpScl. All HpScl cases had a pattern of TDP-43 pathology consistent with FTLTDP Type A, although the pathology was restricted to limbic and inferomedial temporal lobe in about 70% of the cases. Fine neurites in the CA1 sector and subiculum were found in all HpScl cases. Genetic analysis demonstrated the similarity with FTLTDP and HpScl. HpScl cases had the decreased protective allele in *TMEM106B*; however, the increased risk allele in *GRN* did not reach statistical significance. Our findings in LBD support the notion that HpScl in the elderly might share underlying disease mechanisms with FTLTDP and that there is a close relation between HpScl and FTLTDP Type A pathology.

Previous data regarding HpScl in LBD is limited. Yokota et al. [30] reported that none of their 56 LBD cases had HpScl. Their finding cannot be directly compared with our results due to the different pathologic characteristics of cases. In their study, 91% (51/56) of the cases had low Braak NFT stages (0 to IV). In our study only 2.9% of LBD cases with NFT stages 0-IV had HpScl, which may not be significantly different from their study. Of note, they also presented a case that had no HpScl, but abundant TDP-43-positive fine neurites in CA1 sector, which resembles our “pre-HpScl” cases. There was no difference in the frequency of HpScl between transitional and diffuse LBD cases (4.8% vs. 5.5%, $P=0.828$). In addition, the frequency of HpScl in our LBD cases with Braak NFT stages 0-II (2.8%) and NFT stages V-VI (9.1%) was similar to the reported prevalence of “pure-HpScl” [31] and HpScl in AD [5]. These results suggest that Lewy related pathology seems to have no direct association with HpScl.

The following findings support the notion that TDP-43-positive fine neurites might be a precursor lesion for HpScl [8]: 1) all HpScl cases had this pathology in the CA1 sector and subiculum, where selective neuronal loss occurs, and 2) the severity of CA1 fine neurites in Type A cases with and without HpScl was associated with the *TMEM106B* variant. We hypothesized that the *TMEM106B* CC genotype might potentially suppress the progression of fine neurites in the CA1 sector and subiculum, and thus prevent the development of HpScl. We found a subset of LBD cases, which were not diagnosed as having HpScl due to the absence of neuronal loss or the presence of many extracellular NFTs, but had abundant TDP-43-positive CA1 fine neurites and a low frequency of the *TMEM106B* CC genotype. It

is plausible that these cases are on the spectrum of HpScl pathology. We propose a classification of HpScl in LBD based upon neuronal loss, TDP-43 pathology, and extracellular NFTs in the CA1 sector (Fig. 4). “Typical-HpScl” has neuronal loss and TDP-43-positive fine neurites, but no or relatively mild NFTs compared with the degree of neuronal loss. “Pre-HpScl” has none to minimal neuronal loss or extracellular NFTs, but it has abundant TDP-43-positive fine neurites. We propose for the first time that “masked HpScl” may represent HpScl as hidden by many extracellular NFTs, and only recognizable by presence of characteristic TDP-43-positive fine neurites.

In addition, we found a subject with “pre-HpScl” in a non-LBD validation cohort, suggesting that the concept about spectrum of HpScl pathology may also fit with the elderly patients outside the setting of LBD. We are unable to perform genetic studies due to the small size of the validation cohort. We encourage further studies using larger cohorts to clarify genetic associations. A recent genome-wide association study investigating HpScl as an endophenotype also validated the association with *TMEM106B* variant and HpScl in aged individuals [23]. We suggest that TDP-43 immunohistochemistry is needed to diagnose HpScl in the elderly because “pre-HpScl” and “masked-HpScl” cannot be detected by H&E staining. It should also be mentioned that TDP-43-positive fine neurites in the CA1 and subiculum are visible with the immunohistochemistry using non-phosphorylated TDP-43 antibodies (MC2085 and 10782-2-AP) as well as the phosphorylated TDP-43 antibody (pS409/410).

There are a few reports about TDP-43 subtype in LBD. Nakashima-Yasuda et al. reported [22] that the majority (~95%) of TDP-43-positive cases were Type A, and fewer (~5%) were Type B. Arai et al. also reported [3] that all TDP-43-positive cases were Type A if TDP-43 pathology progressed to the neocortex. They also demonstrated common biochemical features of LBD with Type A TDP-43 pathology and FTLTDP Type A based on the immunoblot pattern of phosphorylated C-terminal fragments of TDP-43. Our findings confirmed these findings that Type A is the most frequent subtype in LBD (60%, 101/168); however, the remaining cases were difficult to classify based on Mackenzie scheme for FTLTDP. We found that 26% (43/168) of the TDP-43-positive cases had a unique pattern of TDP-43 pathology; a mixture of NCIs and NFT-like TDP-43 pathology with no or sparse DNIs in the hippocampus and adjacent cortex. These cases had only sparse NCIs in the dentate fascia and no TDP-43 pathology beyond the limbic and inferomedial temporal lobe, which is quite different from the distribution pattern of FTLTDP Type B, which has abundant NCIs in the dentate fascia and frequent pathology in the brainstem including hypoglossal nucleus [11]. Therefore, we called this pattern of TDP-43 pathology as Type B/NFT and distinguished it from Type B. We consider that a new classification criteria for TDP-43 subtype in AD and LBD is needed because the current scheme for FTLTDP does not fit with findings in AD and LBD.

In conclusion, HpScl in LBD is TDP-43 proteinopathy that is similar to FTLTDP-Type A. The presence of HpScl in LBD is associated with greater AD pathology, but not with the distribution of Lewy related pathology. We suggest that TDP-43 immunohistochemistry is needed to diagnosis HpScl in the elderly because TDP-43-positive fine neurites, which are suggested to be a hallmark of HpScl, are not visible with routine histologic methods.

TDP-43 immunohistochemistry expands the spectrum of HpScl by revealing “pre-HpScl” in cases with minimal or no neuronal loss and “masked-HpScl” in cases with neuronal loss and severe NFT pathology. While the present findings lead to a more accurate description of HpScl in the elderly, particularly in LBD, further studies are needed to clarify the clinical significance of HpScl.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Immunohistochemistry for TDP-43 in a hippocampal sclerosis case (Type A). Neuronal cytoplasmic inclusions (NCIs) in the dentate fascia (a). Fine neurites in the CA1 sector (b, c) and subiculum (d). Mixture of NCIs and dystrophic neurites (DNs) in the parahippocampal gyrus (e), inferior temporal gyrus (f), and amygdala (g). Inset in (f) shows a neuronal intranuclear inclusion with a lentiform shape. Sparse TDP-43 pathology in the putamen (h) and substantia nigra (i). Insets in (h) and (i) show a NCI and a DN, respectively. TDP-43 pS409/410 (a-i). Scale bars: a, b, d-i; 50um, C; 200um.

Fig. 2.

Immunohistochemistry for TDP-43 in the case without hippocampal sclerosis (Type B/NFT). Sparse neuronal cytoplasmic inclusions (NCIs) in the dentate fascia (a). Inset in (a) shows a NCI. TDP-43-positive fine neurites are not present in the CA1 sector (b) and subiculum (c). TDP-43 immunoreactivity is shown in some NFTs in the subiculum (c). Inset in (c) shows a NFT-like TDP-43 pathology. Mixture of NCIs and NFT-like TDP-43 pathology in the parahippocampal gyrus with sparse dystrophic neurites (d). TDP-43 pS409/410 (a–d). Scale bars: a–d; 50µm.

Fig. 3.

TDP-43-positive fine neurites in CA1 (a, b) were similar in HpScl-Type A (a) and Not HpScl-Type A High CA1 neurites (b) compared to Not HpScl-Type A Low CA1 neurites (c). CA1 fine neurites are significantly less in all Type A cases (a–c) with *TMEM106B* CC genotypes than in cases with TT ($p=0.002$) or CT genotype ($p=0.018$) (d). Not HpScl-Type A cases with High CA1 fine neurites had low frequency of *TMEM106B* CC genotype similar to HpScl-Type A cases (e). The boxes show median and 25th and 75th percentiles with whisker plots showing 10th and 90th percentiles. Scale bars: a–c; 50µm.

Fig. 4.

We propose a classification of hippocampal sclerosis in LBD based upon neuronal loss, TDP-43 pathology and extracellular NFTs in the CA1 sector (a). “Typical-HpScl” has neuronal loss and TDP-43-positive fine neurites but no or relatively mild NFTs when compared with the degree of neuronal loss (b, e, h). “Pre-HpScl” has none to minimal neuronal loss or extracellular NFTs, but abundant TDP-43-positive fine neurites (c, f, i). “Masked HpScl” has neuronal loss and many extracellular NFTs but also abundant TDP-43-positive fine neurites (d, g, j). Arrows in (d) show extracellular NFTs. Hematoxylin & eosin

(b–d), TDP-43 pS409/410 (e–g), Tau PHF-1 (h–j). Scale bars: b–j; 50um. NL=neuronal loss, eNFTs=extracellular neurofibrillary tangles, H&E=hematoxylin & eosin.

Table 1

Demographics, pathology and genetics of case material

	HpScl n=35	Not HpScl n=634	P-value
Demographic characteristics			
Male	17 (49%)	387 (61%)	0.197
Age at death, years	81 (78, 85)	78 (73, 84)	0.010
Post-mortem findings			
Brain weight, grams	1000 (880, 1140)	1140 (1040, 1260)	<0.001
Braak NFT stage	VI (III, VI)	IV (III, V)	<0.001
Thal amyloid- β phase	5 (4, 5)	4 (3, 5)	0.003
Lewy body subtype			
Diffuse	21 (60%)	359 (57%)	0.828
Limbic	14 (40%)	275 (43%)	
TDP-43 positivity	35 (100%)	133 (21%)	<0.001
Type A	35/35 (100%)	66/133 (50%)	<0.001
Type B/NFT	0/35 (0%)	43/133 (32%)	
Unclassified	0/35 (0%)	24/133 (18%)	
Genetic findings			
<i>TMEM106B</i> , C allele carriers	14/32 (44%)	370/527 (70%)	0.003
<i>GRN</i> , T allele carriers	21/32 (66%)	277/527 (53%)	0.209
<i>APOE</i> , ϵ 4 allele carriers	21/32 (66%)	293/538 (54%)	0.293

All data is displayed as median (25th, 75th range), unless otherwise noted. *TMEM106B* rs1990622 C allele, *GRN* rs5848 T allele, and *APOE* ϵ 4 allele carriers are displayed as number genotyped out of available cases.

Table 2

Frequency of HpScl and TDP-43 pathology by Braak NFT stage and LBD subtype

Braak NFT stage				
	0-II n=141	III-IV n=275	V-VI n=253	P-value
HpScl	4 (2.8%)	8 (2.9%)	23 (9.1%)*	0.002
TDP-43 positivity	11 (7.8%)	57 (21%)**	100 (40%)**	<0.001
LBD subtype				
	Limbic n=289	Diffuse n=380	P-value	
HpScl	14 (4.8%)	21 (5.5%)	0.828	
TDP-43 positivity	63 (22%)	105 (28%)	0.102	

*
P<0.05**
P<0.001 vs. Braak NFT stage 0-II.

Table 3

Semi-quantitative assessment of TDP-43 pathology

	HpSel		Not HpSel				P-value
	Type A n=35		Type A n=19		Type B/NFT n=13		
	TDP-43 score	Positivity	TDP-43 score	Positivity	TDP-43 score	Positivity	
Neuronal cytoplasmic lesions							
Dentate fascia	2 (1, 3)	35/35 (100%)	1 (1, 1)**	18/19 (95%)	0.5 (0, 0.5)**	9/13 (70%)	<0.001
CA1	0.5 (0.5, 1)	30/35 (86%)	0.5 (0.5, 1)	15/19 (79%)	1 (0.25, 1)	10/13 (77%)	0.619
Subiculum	1 (1, 2)	35/35 (100%)	1 (1, 2)	16/19 (84%)	1 (1, 2)	13/13 (100%)	0.550
Parahippocampal gyrus	2 (1, 3)	35/35 (100%)	2 (1, 3)	17/19 (89%)	1 (1, 2)*	13/13 (100%)	0.024
Inferior temporal gyrus	1 (0, 2)	20/27 (74%)	0.5 (0, 2)	9/15 (60%)	0 (0, 0.625)*	3/10 (30%)	0.047
Amygdala	2 (2, 3)	33/33 (100%)	3 (1, 3)	19/19 (100%)	3 (1.5, 3)	13/13 (100%)	0.930
Putamen	0 (0, 5)	9/33 (27%)	0 (0, 0)*	0/19 (0%)	0 (0, 0)*	0/13 (0%)	0.007
Mid-frontal gyrus	0 (0, 0)	6/35 (17%)	0 (0, 0)	3/19 (16%)	0 (0, 0)	0/13 (0%)	0.293
Substantia nigra	0 (0, 0)	4/34 (12%)	0 (0, 0)	1/19 (5%)	0 (0, 0)	0/13 (0%)	0.362
Hypoglossal nucleus	0 (0, 0)	0/26 (0%)	0 (0, 0)	1/17 (6%)	0 (0, 0)	0/12 (0%)	0.327
Neuritic lesions							
Dentate fascia	0.5 (0, 0.5)	24/35 (69%)	0 (0, 0.5)*	6/19 (32%)	0 (0, 0)**	1/13 (8%)	<0.001
CA1	2 (1, 3)	35/35 (100%)	1 (0.5, 2)	19/19 (100%)	0 (0, 0)**	2/13 (15%)	<0.001
Subiculum	1 (0.5, 2)	35/35 (100%)	1 (1, 2)	18/19 (95%)	0.5 (0, 0.5)**	7/13 (54%)	<0.001

	HpScl		Not HpScl				P-value
	Type A n=35		Type A n=19		Type B/NFT n=13		
	TDP-43 score	Positivity	TDP-43 score	Positivity	TDP-43 score	Positivity	
Parahippocampal gyrus	2 (1, 3)	35/35 (100%)	1 (0.5, 3)	17/19 (89%)	0 (0, 0.75)**	5/13 (40%)	<0.001
Inferior temporal gyrus	0.5 (0, 2)	20/27 (74%)	0.5 (0, 1)	8/18 (53%)	0 (0, 0)**	1/10 (10%)	0.004
Amygdala	1 (1, 2)	32/33 (97%)	2 (0.5, 2)	17/19 (89%)	1 (0.25, 1)*	10/13 (77%)	0.020
Putamen	0 (0, 0.25)	8/33 (24%)	0 (0, 0)*	0/19 (0%)	0 (0, 0)	0/13 (0%)	0.013
Mid-frontal gyrus	0 (0, 0)	6/35 (17%)	0 (0, 0)	3/19 (16%)	0 (0, 0)	0/13 (0%)	0.290
Substantia nigra	0 (0, 0.5)	15/34 (44%)	0 (0, 0)	4/19 (21%)	0 (0, 0)*	0/13 (0%)	0.008
Hypoglossal nucleus	0 (0, 0)	0/26 (0%)	0 (0, 0)	0/17 (0%)	0 (0, 0)	0/12 (0%)	1.000
Neuronal nuclear inclusions	n/a	31/35 (89%)	n/a	11/19 (58%)*	n/a	1/13 (8%)**	<0.001

TDP-43-positive cytoplasmic and neuritic lesions were scored separately using a 5 point grading scale (0= none, 0.5= rare, 1 = mild, 2=moderate, 3=severe). TDP-43 score shows median (25th, 75th range) in each brain region. Positivity is number TDP-43-positive out of available cases in each brain region. Abbreviations: n/a., not assessed.

* p<0.05

** P<0.001 vs. HpScl-Type A.

Table 4

Comparison of demographics and genetics by subtype of TDP-43 pathology

	HpScl	Not HpScl		P-value
	Type A n=35	Type A n=66	Type B/NFT n=43	
Male	17 (49%)	32 (48%)	19 (44%)	0.893
Age at death, years	81 (78, 85)	82 (77, 86)	82 (77, 86)	0.953
<i>TMEM106B</i> , C allele carriers	14/32 (44%)	42/55 (76%)*	25/34 (74%)*	0.005
<i>GRN</i> , T allele carriers	21/32 (66%)	32/55 (58%)	19/34 (56%)	0.697
<i>APOE</i> , ε4 allele carriers	21/32 (66%)	42/58 (72%)	21/36 (58%)	0.367

Age at death is displayed as median (25th, 75th range). *TMEM106B* C allele, *GRN* T allele, and *APOE* ε4 allele carriers are displayed as number genotyped out of available cases.

* p<0.05 vs. HpScl-Type A.