Hippocampal sclerosis in frontotemporal dementia : when vascular pathology meets neurodegeneration

Short title: Vascular Pathology and Hippocampal Sclerosis in FTD

Hippocampal Sclerosis in Frontotemporal Dementia: When Vascular Pathology Meets Neurodegeneration

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Abstract

Hippocampal sclerosis (HS) is a common neuropathological finding and has been associated with advanced age, TDP-43 proteinopathy, and cerebrovascular pathology. We analyzed neuropathological data of an autopsy cohort of early-onset frontotemporal dementia patients. The study aimed to determine whether in this cohort HS was related to TDP-43 proteinopathy and whether additional factors could be identified. We examined the relationship between HS, proteinopathies in frontotemporal cortices and hippocampus, Alzheimer disease, cerebrovascular changes, and age. We confirmed a strong association between HS and hippocampal TDP-43, whereas there was a weaker association between HS and frontotemporal lobar degeneration with TDP-43 pathology (FTLD-TDP). Nearly all of the FTLD-TDP cases had TDP-43 pathology in the hippocampus. HS was present in all FTLD-TDP type D cases, in 50% of the FTLD-TDP A cohort and in 6% of the FTLD-TDP B cohort. Our data also showed a significant association between HS and vascular changes. We reviewed the literature on HS and discuss possible pathophysiological mechanisms between TDP-43 pathology, cerebrovascular disease, and HS. Additionally, we introduced a quantitative neuronal cell count in CA1 to objectify the semiquantitative visual appreciation of HS.

Key Words: Cerebrovascular disease, Frontotemporal dementia, Frontotemporal lobar degeneration, FTLD-TDP, Hippocampal sclerosis, TDP-43.

INTRODUCTION

Hippocampal sclerosis (HS) is a neuropathological diagnosis, defined as severe hippocampal neuronal loss and gliosis (1-3). HS is a well-known cause of temporal lobe epilepsy (TLE) and is often associated with drug-resistant seizures. TLE-associated HS comprises 3 types (HS ILAE type 1 to 3), each corresponding with a specific neuropathological correlate (4). In the context of neurodegeneration, another classification scheme is used, with HS divided into 5 subtypes, in which type 4, i.e. neuronal loss and gliosis in CA1 and the subiculum, is mostly referred to as classic HS (5). The clinical features of HS in neurodegenerative conditions are less clear than in epilepsy, with memory loss and poor consolidation of newly learned material as key symptoms (1, 6, 7). Different neurodegenerative disorders have been associated with HS, including frontotemporal dementia (FTD), Alzheimer disease (AD), tauopathies, fused-in-sarcoma (FUS) proteinopathies, and Lewy body disease (8-15).

The strongest association has been observed between TDP-43 proteinopathies and HS, with a prevalence of 70% to 90% of HS cases having TDP-43 pathology (5, 16-18). TDP-43 proteinopathies are a major cause of frontotemporal lobar degeneration (FTLD). FTLD includes a heterogeneous group of proteinopathies, affecting the frontal and/or temporal cortex, thus leading to clinical syndromes which are dominated by a frontal syndrome (behavioral variant FTD, [bvFTD]) or speech disorders (primary progressive aphasia, [PPA]), respectively (19, 20). Since the onset of the disease occurred before the age of 65 in 65% to 80% of the patients, FTLD is considered a leading cause of presenile dementia (21). In the age group between 45 and 64 years, FTLD had a prevalence of 10 to 15 per 100,000 (22), whereas AD in the same age group occurred with a prevalence of 40 per 100,000 (23).

FTLD caused by TDP-43 proteinopathy (FTLD-TDP) can be subclassified into 4 types (A through D), with every type corresponding to a typical pattern of neuronal intracytoplasmic and/or intranuclear TDP-43 aggregates (24). HS was found to be present in 70% of FTLD with TDP-43 inclusions (FTLD-TDP) (16, 25, 26). On the other hand, TDP-43 pathology is often present in the mesotemporal structures of elderly patients; this has recently been described as limbic-predominant age-related TDP-43 encephalopathy (LATE). LATE is assumed to be different from FTLD, with regard to the onset and localization of the TDP-43 pathology (27-30).

Genetic factors are also linked with HS: mutations in the FTD genes *GRN* and *C9orf72* and genetic variations in *TMEM106B* and polymorphisms in *KCNMB2* and *ABCC9* have shown to be risk alleles for HS (17, 27, 31-37). Recent evidence indicates that there is a dose-response relationship and association between carrying an *APOE* Ɛ4 allele in combination with a higher TDP-43 burden and thus higher risk of HS (38, 39).

In addition to neurodegeneration, the aging brain is susceptible to other structural changes and lesions, especially cerebrovascular disease (CVD). Several groups have associated vasculo-ischemic white matter lesions with hippocampal atrophy, or have found arteriosclerotic and anoxic or hypoxic lesions in HS (30, 40-43). Recent research further hints at the link between vascular factors and TDP-43 pathology, with or without HS (44).

Finally, in "pure" HS (prevalence of 0.5% of HS cases), HS is indicated as the only neuropathological substrate for a dementia syndrome. Recent neuropathological assessment, however, suggests that even these rare, pure HS cases are combined with other degenerative changes in other parts of the brain such as vascular pathology or AD (16, 45).

Whereas previous studies focused on the aging population, prone to the co-occurrence of multiple neurodegenerative conditions, we present our findings in an autopsy cohort of early-onset frontotemporal dementia (FTD). Due to the younger age of our cohort, we assumed that neuropathological findings in this cohort would be less extensive compared to those in elderly patients and that fewer confounding pathologies would be present. We hypothesized that hippocampal TDP-43 pathology is necessary for the development of HS, more than FTLD-TDP pathology. Our study aimed to describe whether in this autopsy cohort, HS could be attributed to TDP-43 proteinopathy and whether additional factors could be identified. We examined the relationship between the presence of HS, proteinopathies in frontotemporal cortices, hippocampal proteinopathies, AD, cerebrovascular changes, and age.

MATERIALS AND METHODS

Retrospective Identification of FTD Subjects

This cohort originates from the Neurobiobank of the Institute Born-Bunge (NBB-IBB, FAGG registration number FA190113) located at the University of Antwerp. All recruited and consenting subjects were included according to a standardized brain banking research protocol, and the study was approved by the Medical Ethics Committee of the University Hospital Antwerp (UZA). Cases between 1974 and March 2019 were retrospectively selected.

Cases were included when registered with a clinical diagnosis of onset <65 years of age, "frontotemporal dementia (FTD) with or without motor neuron disorder (MND)", possible or probable behavioral frontotemporal dementia (bvFTD)", "primary progressive aphasia (PPA)", or "behavioral disorders and dementia". Behavioral disorders included apathy, disinhibition with aggression, mental rigidity, lack of insight, distractibility, or lack of personal grooming.

We identified 110 cases with a clinically differential diagnosis of possible or probable bvFTD or PPA. Clinical FTD cases that did not have FTLD but AD or other pathology were also included. We included 8 clinically healthy controls and 8 motor neuron disorder (MND) cases, for a total of 126 cases. Four cases (1 case with Creutzfeldt-Jakob disease, 1 case with Huntington disease, 1 case with diffuse Lewy body disease, 1 case with ceroid lipofuscinosis type 4) were excluded from further analysis after neuropathological assessment resulting in a final cohort of 122 cases in this study.

Neuropathological Assessment

In all our patients and controls, the brain was dissected after a mean formalin fixation (10%-12%) period of 6 to 8 weeks and specific regions were selected and paraffin-embedded. The following brain regions were regionally dissected based on previous protocols (46): Brodmann area (BA) 4 (gyrus precentralis), BA 6 (gyrus frontalis superior), BA 8 (frontal eye fields), BA 11 (gyrus rectus), BA 24 (gyrus cinguli), BA 22 (gyrus temporalis superior), anterior and medial hippocampus, gyrus parahippocampalis, amygdala, BA 7 (lobulus parietalis superior), BA 17 (area striata), cerebellum, thalamus, neostriatum, pallidum, mesencephalon, pons and medulla oblongata.

White matter and vascular changes were examined in the frontal cortex, hippocampus, temporal neocortex and adjacent white matter, area striata, and cerebellum. Deep white matter was examined in the internal capsule (crus anterior and posterior), at the level of the central nucleus of the thalamus, and at the level of the maximal diameter of the external and internal part of the pallidum.

Histological analysis was performed using hematoxylin and eosin, Cresyl violet and Klüver-Barrera. Immunohistochemistry was performed with antibodies against ubiquitin (polyclonal rabbit anti-ubiquitin REF 20458; Dako, Glostrup, Denmark), hyperphosphorylated tau (AT8; Innogenetics, Zwijnaarde, Belgium), β-amyloid (4G8; Signet, Dedham, MA), α-synuclein (polyclonal anti-α-synuclein antibody REF S3062; Sigma Aldrich, St Louis, MO), TDP-43 (TDP-43 polyclonal antibody REF 10782-2-AP; Proteintech Group, Inc., Chicago, IL), FUS (FUS/TLS monoclonal antibody REF 60160-1-Ig; Proteintech Group, Inc.), p62 (purified monoclonal mouse anti-p62 ligand REF 610833; BD Diagnostics, Erembodegem, Belgium).

Hippocampal Sclerosis

The anterior part of the hippocampus was coronally-sliced caudal to the amygdala, whereas the medial part of the hippocampus was sliced at the level of the lateral geniculate body (5 µm). Both sections were semi-quantitatively analyzed, and the slice of the medial hippocampus was also quantitatively analyzed. In this slice, the region of interest was identified and independently rated by 2 neuropathologists (JJM and AS).

CA1 was neuroanatomically identified using the descriptions of Duvernoy et al (47). As proposed by Rauramaa et al (5), HS was diagnosed if type 4 pathology, i.e. neuronal loss and gliosis in CA1, was present. Because gliosis is difficult to assess, we focused on neuronal loss. Cases with severe neuronal loss in CA1 due to AD were not considered to have HS. The semiquantitative analysis included a visual rating based on neuronal loss in CA1 and/or the subiculum (hippocampal sclerosis absent or present). Normal visual cell density was given a score of “0”, i.e. no hippocampal sclerosis, whereas severe neuronal cell loss was rated “1”. Cases were evaluated by 2 separate raters, blinded from each other (JJM and AS) (Cohen’s K: 0.80). When there was a disagreement, cases were individually discussed until consensus was reached. To determine whether these qualitative data were quantifiable, the slices were scanned using a Zeiss Axio Imager M2 microscope. Next, the pyramidal layer of CA1 was delineated using the AxioVison software (Zeiss), where pyramidal neurons were manually counted.

Proteinopathies

Every case was examined for beta-amyloid pathology, neurofibrillary tangles, Lewy bodies, FUS inclusions and TDP-43 pathology with a specific focus on the hippocampal regions.

The proteinopathy causative for the clinical syndrome was identified. The National Institute on Aging-Alzheimer’s Association Criteria were used for AD neuropathology diagnosis used (48); this was based on a Braak score for hyperphosphorylated tau pathology (49), Thal stages for amyloid

pathology (50), and CERAD staging of neuritic plaques (51). To assess the likelihood of clinical correlation, we referred to the NIA-Reagan Criteria (52).

Cases were divided into 4 groups depending on the likelihood for clinical correlation: the 4 groups of "Not", "Low", "Intermediate" or "High" correlation with clinical phenotype were evaluated based on the presence of HS. Lewy body pathology classification of McKeith et al was used (53), whereas we applied the Mackenzie criteria for TDP-43 proteinopathies (24).

Cerebrovascular Pathology and Cerebral Amyloid Angiopathy

Staging of cerebrovascular pathology was based on Deramecourt et al (54). Arteriosclerotic changes, perivascular hemosiderin deposits and dilation of the perivascular space together with hemorrhages and micro or lacunar infarcts were representative of CVD. Three groups were created: no or mild vascular alterations (score 0 or 1 of Deramecourt); moderate alterations (=score 2 of Deramecourt) or severe vascular changes (score 3 or 4 of Deramecourt), hemorrhages or infarctions. As infarctions are end-stage evidence of cerebrovascular pathology, infarctions in basal ganglia were also separately analyzed.

Cerebral amyloid angiopathy (CAA) pathology was scored as suggested by Love et al as follows: 0: no CAA; 1: sporadic (trace of occasional vessel affected); 2: moderate (one of a few vessels circumferentially affected); and 3: severe (widespread involvement of circumferentially affected vessels) (55). Cerebrovascular pathology was independently rated by 2 assessors blinded from each other (MP and AS) (Cohen's K: 0.527).

Age

Age of death was included as a continuous variable. However, as many studies include >80 years old as a specific advanced age group, this additional variable was also created.

Statistical Analysis

The presence of HS was the primary outcome measure in this study. The discriminating ability of the quantitative neuronal cell count to predict HS was investigated through ROC curve analysis (AUC). Mean neuronal cell count in the HS group versus the non-HS group was evaluated using an independent t-test.

Exploratory data analysis was performed by reporting the number of HS cases per level of categorical variables including: (i) FTLD-TDP, (ii) FTLD- TPD subtype, (iii) TDP-43 in hippocampus, (iv) p62 pathology in hippocampus, (v) advanced age (>80), (vi) Thal score, (vii) Braak score, (viii) AD neuropathological changes based on likelihood for clinical correlation, (ix) severity of vascular

pathology in the deep white matter and basal ganglia, (x) infarctions in deep white matter and basal ganglia, (xi) cerebral amyloid angiopathy, (xii) age as a continuous variable, and (xiii) Deramecourt total score.

Simple logistic regression analyses were performed to investigate the influence of different variables on outcome measure. In the multivariable logistic regression analysis, associations between relevant variables and outcome measures were examined. Likelihood ratio tests were used due to small sample sizes. Estimated odds ratios and their 95% profile likelihood confidence intervals were reported. P values <0.05 were considered significant. All statistical analyses were performed in SPSS 25 (IBM SPSS version 25 Software, IBM, Corp., Armonk, NY).

RESULTS

Description of Study Population

Our final study cohort consisted of 122 cases, of whom 73 showed FTLD or FTLD-MND pathology (68 FTLD and 5 FTLD-MND cases), whereas 49 cases had a non-FTLD-MND neuropathology diagnosis: 28 cases with AD as primary neuropathological diagnosis, 5 cases with severe cerebral vascular pathology (VAD), 8 patients with MND (without FTLD) and 8 controls without major neuropathological findings (Table 1). Gender differences were evenly distributed over neuropathological subgroups. Mean age of death was 66.9 years (from 34 to 92 years old), 14 cases were classified as advanced age (>80 years old) (Table 1)

Neuropathology

FTLD Proteinopathies

Of the 73 FTLD subjects, 66% had FTLD-TDP, with FTLD-TDP type B being the most prevalent (41%). The second most common subtype was FTLD-TDP type A (16%), followed by FTLD-TDP type D pathology (6%). Only 1 subject had FTLD-TDP type C. The 5 subjects with FTLD-MND-TDP had TDP-43 type B pathology. The remaining FTLD population was either diagnosed with a tauopathy: PSP/CBD tauopathy (20.5%), Pick disease (5.5%), GGT (2.7%) and AGD (1 case), or with FTLD-FUS (2 cases), or with FTLD-UPS (1 case) (Fig. 1A-C). In the non-FTLD counterparts, 7 out of 8 MND subjects had a TDP-43 proteinopathy, while 1 subject had MND pathology related to *SOD1* mutation with ubiquitinated inclusions in the motor neuron cells.

Hippocampal Proteinopathies

Hippocampal TDP-43 pathology was found in 60.0% of all cases. Of these cases with hippocampal TDP-43, 60% were associated with FTLD-TDP (27.9% FTLD-TDP A, 60.5% FTLD-TDP B, 2.3% FTLD-TDP C, 9.3% FTLD-TDP D). In the remaining 40% of hippocampal TDP-43 cases without associated FTLD-TDP, AD was present in 48%, whereas 31% had PSP/CBD. FTLD-UPS, GGT, VAD, Pick, MND-TDP and MND-Ubi were present in 3.4% (Tables 1 and 2). None of the control cases had any hippocampal TDP-43. In 5 cases, mesotemporal TDP-43 pathology was suggestive for LATE. In the hippocampal TDP-43-negative group (48 of 120 cases), 6% (n = 3) had p62-immunoreactive, tau-negative and α-synuclein-negative neuronal cytoplasmic inclusions in the hippocampus.

Vascular Changes

On the whole, 28% of cases had none to mild CVD in the basal ganglia and deep white matter, whereas 47.4% had moderate CVD, and 25.0% had severe CVD. Focusing on the presence of

infarctions as the end stage of CVD, we found infarctions in the deep white matter and basal ganglia in 16.4% of cases (n = 19). Infarctions that were spread over the entire brain were found in 24.1% of cases (n = 28). Mean Deramecourt total score was 5.4 out of 20 (SD 2.6). Minimum score was 0 out of 20, and maximum score was 14 out of 20. Six cases were excluded from analysis, as there was no possibility to score thalamus and/or basal ganglia.

Cerebral Amyloid Angiopathy

8.8% (n = 10) had sporadic CAA changes, 11.4% (n = 13) had circumferential CAA pathology, 14% (n = 16) had widespread CAA. 65.8% (n = 75) of the patient population did not have any CAA. Twelve cases were excluded due to technical factors, such as absence of sampling.

Alzheimer Disease

In total, 71 cases were identified with AD pathology: a high correlation (see [52]) with clinical phenotype was present in 14.8% ($A_3B_3C_{2/3}$), whereas 12.3% had intermediate AD pathology ($A_{1/2}B_{2/3}C_{2/3}$ to $A_3B_2C_{2/3}$) and in 31.1% of cases, the AD pathology was considered to be low ($A_{1/2}B_{1/2/3}C_1$ to $A_{2/3}B_1C_{1/2/3}$). In the 28 cases that had AD as a primary diagnosis, 18 had a high correlation with clinical phenotype (i.e. severe AD pathology), 9 had intermediate AD pathology and 1 subject had only mild AD pathology. In 43 cases, AD pathology occurred as coexisting pathology (i.e. considered not to be a factor contributing to the clinical phenotype) including in 4 out of 8 clinically healthy controls. In most cases, AD pathology was considered to be low.

Hippocampal Sclerosis

In total, 24 out of 122 patients had HS (Fig. 2). Out of these 24 cases, 18 HS subjects had FTLD(-MND), of which FTLD-(MND)-TDP was present in 13 of HS cases. Three HS subjects had FTLD-tau, 2 HS subjects had FTLD-FUS and 6 HS subjects had AD (Table 2).

Association Between Proteinopathies, Vascular Disease, Age and HS

There was no association between FTLD-(MND)-TDP and HS ($p = 0.101$). Six out of 12 subjects with FTLD-TDP A had HS, whereas only 2 out of 30 subjects with FTLD-TDP B had HS. HS was not present in 1 FTLD-TDP C case, but all 5 subjects with FTLD-TDP D had HS ($p < 0.001$) (Table 3). In the FTLD-Tau group, no associations could be made between the tauopathies and HS. The 2 subjects with FTLD-FUS had HS. In the MND, VAD and control groups, no HS was present. (Table 1). In the 28 subjects with AD, 6 had HS (21.4%). Addition of the AD pathology to the regression model did not improve the model nor the prediction of the presence of HS ($p = 0.902$).

There was a strong association between hippocampal TDP-43 pathology and HS (p = 0.002), regardless of neuropathological primary diagnosis, with hippocampal TDP-43 present in 20 of 24 HS cases (Tables 2 and 3). Additionally, p62-positive, TDP-43-negative hippocampal inclusions were not associated with HS (p = 0.246).

Vascular changes in deep white matter and basal ganglia were analyzed in 116 cases. There was an association between severe vascular changes and HS presence in 9 out of 29 cases (p = 0.034). In the group with no to mild vascular changes, 2 out of 32 subjects had HS (6.3%), whereas 11 out of 55 subjects with moderate vascular changes (20.0%) had HS, and 9 out of 29 subjects with severe vascular changes had HS (31.0%).

There was no association between infarctions at the level of deep white matter and basal ganglia and thalamus, and HS (p = 0.694). The Deramecourt total score in the “no HS” group was 5.48 (standard deviation of 2.748) versus 5.14 (standard deviation of 1.957) in the “HS” group, showing no value as a predictor of HS (p = 0.591) (Table 3). Cerebral amyloid angiopathy was present in 39 of 114 cases. Sixteen of 39 demonstrated severe CAA, of which 3 had HS (p = 0.156).

In our study population, the mean age in the “no HS” group was 67.16 (10.218) and in the “HS” group was 65.58 (12.796), showing no association between HS and age (p = 0.518). In the 14 cases over >80 years, 3 had HS (p = 0.862). We could not find any significant predictive value of advanced age (>80), hippocampal TDP-43, severe vascular pathology or CAA.

Because hippocampal TDP-43, FTLD-TDP type D and severe vascular pathology in deep white matter tracts were considered significant predictor variables for HS in this univariable logistic regression analysis model, we consequently examined these variables following multiple variable regression analysis. However, after inclusion of these variables, only hippocampal TDP-43 and FTLD-TDP subtype D remained significant predictive factors for HS (Table 4).

Neuronal Count in CA1

The visual score was compared to the absolute neuronal number count in CA1. A total of 115 out of 122 cases were completed. As expected, the mean neuronal number in CA1 was higher in the group without hippocampal sclerosis than in the group with neuronal loss in CA1. The mean pyramidal neuronal cell count in CA1 in the “no HS” group was 586.11 on average (95% CI: 537.59; 634.63), whereas in the “HS” group, the mean number was 249.77 (95% CI: 177.09; 322.46) (Fig. 3A).

With these results, it could be assumed that our semiquantitative visual rating scale was sufficient to discriminate whether HS was present or not. This was also illustrated with an AUC (area under the

curve) value of 0.90 (95% CI: 0.83; 0.97), thus assuming that neuronal counting in CA1 has sufficient discriminatory power to identify the presence of HS (Fig. 3B; sensitivity: 82%, specificity: 82%, Youden Index: 0.635).

DISCUSSION

In this autopsy cohort of mostly early-onset dementia patients, we confirmed the strong association between HS and hippocampal TDP-43, in addition to that with FTLD-TDP type D, and to a lesser extent with FTLD-TDP type A. Nearly all of the subjects with FTLD-TDP had TDP-43 pathology in the hippocampus (Table 2), while 6 out of 12 in the FTLD-TDP A cohort, 2 out of 30 in the FTLD-TDP B cohort and all subjects in the FTLD-TDP D cohort had HS (n = 5). Interestingly, our data also showed an association between HS and severe vascular changes in the deeper white matter and basal ganglia.

Hippocampal TDP-43 Pathology and HS

The specific concept of "Pathoklise" was proposed by Vogt and Vogt, suggesting that intrinsic hippocampal neuronal cell characteristics lead to the susceptibility of cell death (56). In 1986, Rothman and Olney showed that the neurons of CA1 have more glutamate receptors compared to the other more resilient hippocampal neurons. Disease states such as epilepsy have been known to induce an overabundance of glutamate in the synapse, which can be toxic due to postsynaptic Ca^{2+} influx. Neurons that are richer in glutamate receptors are more prone to cell death, referred to as glutamate excitotoxicity (57). Additionally, the more vulnerable CA1 neurons have less Ca^{2+} buffering capacity, due to small concentrations or even absence of calbindin and chromogranin A compared to more resistant neurons (58). Therefore, a poor ability to protect against the excitotoxicity effect of glutamate and Ca^{2+} influx, is the pathogenetic mechanism of HS in conditions such as epilepsy.

In neurodegeneration, the pathogenesis of HS is not completely understood. The link between glutamate excitotoxicity and neurodegeneration is not clear. Other pathways have been suggested to have an impact on neuronal loss in CA1, such as an altered inhibitory input (59). Different groups also hypothesized that CA1 neurons have an impaired degradation of proteins, thus leading to an accumulation of possible toxic protein deposits (60-62).

The association between TDP-43 pathology and neuronal loss has been explained by a pathophysiological mechanism through reactive oxygen species (ROS) and a deficient or dysfunctional heat shock response. This leads to an accumulation of TDP-43 in stress granules and the subsequent formation of toxic TDP-43 oligomers (63, 64). We suggest that this mechanism occurs in every cell affected by TDP-43 inclusions, and depending on intrinsic cell qualities, neurodegeneration and sclerosis occur, as seen in CA1.

A relation between hippocampal TDP-43 pathology and HS in epilepsy has not been found, nor a relation between FTLD and epilepsy (65, 66), but recent data in the epilepsy research field suggest a cognitive decline in patients with TLE, with tau pathology being present in TLE-associated HS cases. Also, alpha-synuclein has been reported to be higher in cerebrospinal fluid and serum of patients with refractory epilepsy (67, 68). In our study, we did not find any relation between HS and hippocampal tauopathy nor synucleinopathy. Furthermore, when looking at the severity of the AD pathology, we could not find any association with HS. No link between HS and cerebral amyloid angiopathy was found.

HS and Cerebrovascular Pathology

The significant association between HS and cerebrovascular pathology, suggests a vascular contribution to the development of HS. This was previously hypothesized by Spielmeyer (69) and Uchimura (quoted by Boling et al [70]) and recently reproduced by Neltner et al and Zarow et al (41, 43). Spielmeyer stated that CA1, being most compromised in HS, has a suboptimal vessel supply compared to neighboring parts, as it is located in a watershed area between the anterior choroidal artery and posterior cerebral arteries (71). Recently, the differences between the hippocampal and neocortical vasculature, with a challenging small number of capillary anastomoses in the hippocampus and high degree of interindividual variations of hippocampal blood supply, have also been proposed as a potential underlying predisposing factor following 7-Tesla MRI studies (72, 73). Next to local ischemic events, other groups have suggested a systemic hypoxia/ischemia hypothesis, while still others have assumed a disruption of the blood-brain barrier (74-76).

In 2012, a staging system was developed to rate cerebrovascular changes, which allowed standardizing and semi-quantifying cerebral vascular changes (54). This staging scheme was used to score cerebrovascular pathology in frontal and temporal lobes, as well as deep white matter at the level of the thalamus and basal ganglia. Because previous studies described a correlation between arteriolosclerosis and HS (41), between deep white matter hyperintensities (WMH) and hippocampal volume (40), and between deep WMH and hippocampal regional cerebral blood flow (77), we focused on the cerebrovascular pathology in basal ganglia and deep white matter.

Using this staging system, we did not find any association between the total vascular score and HS, but we did find a relation between HS and the severe cerebrovascular alterations in deeper white matter tracts and basal ganglia, i.e. arteriolosclerosis, perivascular demyelination, and perivascular hemosiderin deposition. These findings are in line with the findings of Neltner et al (41).

In ischemic conditions, CA1 neurons are more vulnerable compared to other neurons, a phenomenon which has been explained by the “calpain-cathepsin” hypothesis. In CA1 neurons, the presence of ROS and altered Ca^{2+} homeostasis, as a result of hypoxia or ischemia, leads to the carbonylation of heat shock protein 70 (HSP70), inducing a destabilization of the lysosome. The carbonylated HSP70 is cleaved by calpain, which causes a loss of integrity of the lysosomal membrane. Due to leakage of the lysosomal membrane, cathepsin spills into the cell cytoplasm, which in turn induces the degeneration of CA1 neurons. In other neurons this calpain-cathepsin axis does not exist, probably because of less mobilization of intracellular calcium, compared to CA1 neurons (75, 78).

Cerebrovascular Pathology and TDP-43

In 2007, Lee et al (66) did not find any TDP-43 pathology in anoxic and ischemic lesions. In contrast to these findings, Katsumata et al (44) found an association between arteriolosclerosis pathology and TDP-43 in the amygdala and entorhinal cortex in persons without *APOE* Ɛ4 homozygosity. Additionally, in 2010, Geser et al found that certain chronic vascular diseases have the potential to drive the phosphorylation and misfolding of TDP-43 (79). However, further data exploring possible pathways between TDP-43 and vascular pathology are lacking, and also the design of our study does not allow any statements about this possible interaction.

Advanced Age

Whereas different studies and reviews have been published emphasizing the association between HS and advanced age (42, 80), we could not find any association between these 2 variables. However, the difference between our study and previously published reports is that the mean age in our group was 66.9 years, whereas, to our knowledge, the average age in other studies was higher. As the focus of our study was on early-onset dementia patients, we can assume that our group of elderly patients was too small to draw conclusions about any relation between advanced age and HS. Even if we consider the relatively long disease course and/or advanced age of some cases, there was no evident association with HS, unlike other publications (18). Nevertheless, based on our results, the presence of HS also needs to be considered in early-onset dementias.

Neuronal Count in CA1

Additionally, we were able to associate the semiquantitative visual diagnosis of HS with a quantitative neuronal cell count in CA1. This implies that the neuronal cell loss is sufficient for diagnosing HS. If future research identifies pyramidal neurons in CA1 and the subiculum, we suggest that quantitative measurement is preferential in the diagnosis of hippocampal sclerosis.

Limitations

Our study has several limitations. We were not able to make any conclusion concerning symmetrical or asymmetrical hippocampal involvement, as our research protocol includes that only the right hemisphere is processed for neuropathological analysis, whereas the left is stored at -80°C for further genetic or neurochemical analyses. Furthermore, examining the right brain hemisphere could be a cause of underdiagnosing HS, as TDP-43 pathology mostly occurs bilaterally in the hippocampus, whereas one should keep in mind that in 40% to 55% of cases, HS is only asymmetrical, inducing a false negative detection of HS in approximately 25% of HS cases (11, 43). This could induce an underestimation of the correlation between TDP-43 and HS. Overall, the small number of cases in our study makes our statistical analyses tenuous. However, it allows us to detect the tendency that HS is strongly associated with TDP-43 pathology in the hippocampus, and we assume that small vessel changes play an additional role, as previously detected in older cohorts (41). Our study lacked a systematic genetic analysis, due to the retrospective concept of our study.

Conclusions

We were able to confirm the strong relationship between hippocampal TDP-43 pathology and HS. We found that the FTLD-TDP type D and to a lesser extent type A cases more frequently showed HS. Interestingly, the odds for HS were significantly higher when subjects had more severe vascular changes in deeper white matter and basal ganglia.

Due to the concept of this autopsy study, with HS as endpoint, we cannot make a statement about any relation between cerebrovascular disease and TDP-43 pathology. Referring to our data, we assume that cerebrovascular disease does not have a direct impact on development of HS. Possible mechanisms of action could include a double hit phenomenon with hippocampal TDP-43 pathology and cerebrovascular pathology leading to HS, or cerebrovascular pathology acting as a modulatory contributing factor on TDP-pathology and thus HS. An upstream effect inducing cerebrovascular pathology, TDP-43 pathology and HS separately from each other, can neither be ruled out.

Furthermore, a quantitative neuronal cell count in CA1 was introduced to objectify the semiquantitative visual appreciation of HS, implying that neuronal cell loss in CA1 is sufficient for diagnosing HS. We propose that quantitative measurement is preferential in the diagnosis of hippocampal sclerosis.

We suggest that research should also focus on the assessment of hippocampal functioning in the workup of FTD patients, through imaging and neuropsychological testing, as this could be a biomarker of TDP-43 pathology and vascular risk factors. Additionally, the systematic gene analysis

with screening of the known FTLD-TDP associated genes – but also of the HS risk genes – may be the next step forward in identifying and clarifying dementia-specific pathophysiological mechanisms of TDP-43 leading to HS.

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Legends to

Figure 1. (**A**) Pie chart with percentages of proteinopathy associated with clinical syndrome. (**B**) Pie chart demonstrating percentages of FTLD-TDP subtypes. (**C**) Pie chart with percentages of FTLD-tau subtypes. FTLD: frontotemporal lobar degeneration; FTLD-tau: frontotemporal lobar degeneration with tauopathy; FTLD other : collection of all non-tau-non-TDP FTLD cases; FTLD-TDP: frontotemporal lobar degeneration with TDP-43 proteinopathy; MND: (pure) motor neuron disease without FTLD; AD: Alzheimer disease; VAD: vascular disease; mixed: mixed dementia VAD + AD. AGD: argyrophilic grain disease; CBD: corticobasal degeneration; GGT: globular glial tauopathy; PSP: progressive supranuclear palsy

Figure 2. Hippocampal sclerosis with neuronal loss in CA1 demonstrated by CV stain. (**A**) AD. (**B**) FTLD-tau (Pick disease). (**C**) FTLD-FUS. (**D**) FTLD-TDP type A. (**E**) FTLD- TDP type B. (**F**) FTLD-TDP type D. Note the relatively mild neuronal loss in CA1 in **E** (*CA1).

Figure 3. (**A**) Box plots show the mean cell count in CA1 in the group without HS ('no') versus the group with HS ('HS'). As expected, the mean neuronal number in CA1 is lower in the 'HS' group. (**B**) ROC curve with AUC of cell count in CA1 plotted against HS.

Short title: Vascular Pathology and Hippocampal Sclerosis in FTD

Hippocampal Sclerosis in Frontotemporal Dementia: When Vascular Pathology Meets Neurodegeneration

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Abstract

Hippocampal sclerosis (HS) is a common neuropathological finding and has been associated with advanced age, TDP-43 proteinopathy, and cerebrovascular pathology. We analyzed neuropathological data of an autopsy cohort of early-onset frontotemporal dementia patients. The study aimed to determine whether in this cohort HS was related to TDP-43 proteinopathy and whether additional factors could be identified. We examined the relationship between HS, proteinopathies in frontotemporal cortices and hippocampus, Alzheimer disease, cerebrovascular changes, and age. We confirmed a strong association between HS and hippocampal TDP-43, whereas there was a weaker association between HS and frontotemporal lobar degeneration with TDP-43 pathology (FTLD-TDP). Nearly all of the FTLD-TDP cases had TDP-43 pathology in the hippocampus. HS was present in all FTLD-TDP type D cases, in 50% of the FTLD-TDP A cohort and in 6% of the FTLD-TDP B cohort. Our data also showed a significant association between HS and vascular changes. We reviewed the literature on HS and discuss possible pathophysiological mechanisms between TDP-43 pathology, cerebrovascular disease, and HS. Additionally, we introduced a quantitative neuronal cell count in CA1 to objectify the semiquantitative visual appreciation of HS.

Key Words: Cerebrovascular disease, Frontotemporal dementia, Frontotemporal lobar degeneration, FTLD-TDP, Hippocampal sclerosis, TDP-43.

INTRODUCTION

Hippocampal sclerosis (HS) is a neuropathological diagnosis, defined as severe hippocampal neuronal loss and gliosis (1-3). HS is a well-known cause of temporal lobe epilepsy (TLE) and is often associated with drug-resistant seizures. TLE-associated HS comprises 3 types (HS ILAE type 1 to 3), each corresponding with a specific neuropathological correlate (4). In the context of neurodegeneration, another classification scheme is used, with HS divided into 5 subtypes, in which type 4, i.e. neuronal loss and gliosis in CA1 and the subiculum, is mostly referred to as classic HS (5). The clinical features of HS in neurodegenerative conditions are less clear than in epilepsy, with memory loss and poor consolidation of newly learned material as key symptoms (1, 6, 7). Different neurodegenerative disorders have been associated with HS, including frontotemporal dementia (FTD), Alzheimer disease (AD), tauopathies, fused-in-sarcoma (FUS) proteinopathies, and Lewy body disease (8-15).

The strongest association has been observed between TDP-43 proteinopathies and HS, with a prevalence of 70% to 90% of HS cases having TDP-43 pathology (5, 16-18). TDP-43 proteinopathies are a major cause of frontotemporal lobar degeneration (FTLD). FTLD includes a heterogeneous group of proteinopathies, affecting the frontal and/or temporal cortex, thus leading to clinical syndromes which are dominated by a frontal syndrome (behavioral variant FTD, [bvFTD]) or speech disorders (primary progressive aphasia, [PPA]), respectively (19, 20). Since the onset of the disease occurred before the age of 65 in 65% to 80% of the patients, FTLD is considered a leading cause of presenile dementia (21). In the age group between 45 and 64 years, FTLD had a prevalence of 10 to 15 per 100,000 (22), whereas AD in the same age group occurred with a prevalence of 40 per 100,000 (23).

FTLD caused by TDP-43 proteinopathy (FTLD-TDP) can be subclassified into 4 types (A through D), with every type corresponding to a typical pattern of neuronal intracytoplasmic and/or intranuclear TDP-43 aggregates (24). HS was found to be present in 70% of FTLD with TDP-43 inclusions (FTLD-TDP) (16, 25, 26). On the other hand, TDP-43 pathology is often present in the mesotemporal structures of elderly patients; this has recently been described as limbic-predominant age-related TDP-43 encephalopathy (LATE). LATE is assumed to be different from FTLD, with regard to the onset and localization of the TDP-43 pathology (27-30).

Genetic factors are also linked with HS: mutations in the FTD genes *GRN* and *C9orf72* and genetic variations in *TMEM106B* and polymorphisms in *KCNMB2* and *ABCC9* have shown to be risk alleles for HS (17, 27, 31-37). Recent evidence indicates that there is a dose-response relationship and association between carrying an *APOE* Ɛ4 allele in combination with a higher TDP-43 burden and thus higher risk of HS (38, 39).

In addition to neurodegeneration, the aging brain is susceptible to other structural changes and lesions, especially cerebrovascular disease (CVD). Several groups have associated vasculo-ischemic white matter lesions with hippocampal atrophy, or have found arteriosclerotic and anoxic or hypoxic lesions in HS (30, 40-43). Recent research further hints at the link between vascular factors and TDP-43 pathology, with or without HS (44).

Finally, in "pure" HS (prevalence of 0.5% of HS cases), HS is indicated as the only neuropathological substrate for a dementia syndrome. Recent neuropathological assessment, however, suggests that even these rare, pure HS cases are combined with other degenerative changes in other parts of the brain such as vascular pathology or AD (16, 45).

Whereas previous studies focused on the aging population, prone to the co-occurrence of multiple neurodegenerative conditions, we present our findings in an autopsy cohort of early-onset frontotemporal dementia (FTD). Due to the younger age of our cohort, we assumed that neuropathological findings in this cohort would be less extensive compared to those in elderly patients and that fewer confounding pathologies would be present. We hypothesized that hippocampal TDP-43 pathology is necessary for the development of HS, more than FTLD-TDP pathology. Our study aimed to describe whether in this autopsy cohort, HS could be attributed to TDP-43 proteinopathy and whether additional factors could be identified. We examined the relationship between the presence of HS, proteinopathies in frontotemporal cortices, hippocampal proteinopathies, AD, cerebrovascular changes, and age.

MATERIALS AND METHODS

Retrospective Identification of FTD Subjects

This cohort originates from the Neurobiobank of the Institute Born-Bunge (NBB-IBB, FAGG registration number FA190113) located at the University of Antwerp. All recruited and consenting subjects were included according to a standardized brain banking research protocol, and the study was approved by the Medical Ethics Committee of the University Hospital Antwerp (UZA). Cases between 1974 and March 2019 were retrospectively selected.

Cases were included when registered with a clinical diagnosis of onset <65 years of age, "frontotemporal dementia (FTD) with or without motor neuron disorder (MND)", possible or probable behavioral frontotemporal dementia (bvFTD)", "primary progressive aphasia (PPA)", or "behavioral disorders and dementia". Behavioral disorders included apathy, disinhibition with aggression, mental rigidity, lack of insight, distractibility, or lack of personal grooming.

We identified 110 cases with a clinically differential diagnosis of possible or probable bvFTD or PPA. Clinical FTD cases that did not have FTLD but AD or other pathology were also included. We included 8 clinically healthy controls and 8 motor neuron disorder (MND) cases, for a total of 126 cases. Four cases (1 case with Creutzfeldt-Jakob disease, 1 case with Huntington disease, 1 case with diffuse Lewy body disease, 1 case with ceroid lipofuscinosis type 4) were excluded from further analysis after neuropathological assessment resulting in a final cohort of 122 cases in this study.

Neuropathological Assessment

In all our patients and controls, the brain was dissected after a mean formalin fixation (10%-12%) period of 6 to 8 weeks and specific regions were selected and paraffin-embedded. The following brain regions were regionally dissected based on previous protocols (46): Brodmann area (BA) 4 (gyrus precentralis), BA 6 (gyrus frontalis superior), BA 8 (frontal eye fields), BA 11 (gyrus rectus), BA 24 (gyrus cinguli), BA 22 (gyrus temporalis superior), anterior and medial hippocampus, gyrus parahippocampalis, amygdala, BA 7 (lobulus parietalis superior), BA 17 (area striata), cerebellum, thalamus, neostriatum, pallidum, mesencephalon, pons and medulla oblongata.

White matter and vascular changes were examined in the frontal cortex, hippocampus, temporal neocortex and adjacent white matter, area striata, and cerebellum. Deep white matter was examined in the internal capsule (crus anterior and posterior), at the level of the central nucleus of the thalamus, and at the level of the maximal diameter of the external and internal part of the pallidum.

Histological analysis was performed using hematoxylin and eosin, Cresyl violet and Klüver-Barrera. Immunohistochemistry was performed with antibodies against ubiquitin (polyclonal rabbit anti-ubiquitin REF 20458; Dako, Glostrup, Denmark), hyperphosphorylated tau (AT8; Innogenetics, Zwijnaarde, Belgium), β-amyloid (4G8; Signet, Dedham, MA), α-synuclein (polyclonal anti-α-synuclein antibody REF S3062; Sigma Aldrich, St Louis, MO), TDP-43 (TDP-43 polyclonal antibody REF 10782-2-AP; Proteintech Group, Inc., Chicago, IL), FUS (FUS/TLS monoclonal antibody REF 60160-1-Ig; Proteintech Group, Inc.), p62 (purified monoclonal mouse anti-p62 ligand REF 610833; BD Diagnostics, Erembodegem, Belgium).

Hippocampal Sclerosis

The anterior part of the hippocampus was coronally-sliced caudal to the amygdala, whereas the medial part of the hippocampus was sliced at the level of the lateral geniculate body (5 µm). Both sections were semi-quantitatively analyzed, and the slice of the medial hippocampus was also quantitatively analyzed. In this slice, the region of interest was identified and independently rated by 2 neuropathologists (JJM and AS).

CA1 was neuroanatomically identified using the descriptions of Duvernoy et al (47). As proposed by Rauramaa et al (5), HS was diagnosed if type 4 pathology, i.e. neuronal loss and gliosis in CA1, was present. Because gliosis is difficult to assess, we focused on neuronal loss. Cases with severe neuronal loss in CA1 due to AD were not considered to have HS. The semiquantitative analysis included a visual rating based on neuronal loss in CA1 and/or the subiculum (hippocampal sclerosis absent or present). Normal visual cell density was given a score of “0”, i.e. no hippocampal sclerosis, whereas severe neuronal cell loss was rated “1”. Cases were evaluated by 2 separate raters, blinded from each other (JJM and AS) (Cohen’s K: 0.80). When there was a disagreement, cases were individually discussed until consensus was reached. To determine whether these qualitative data were quantifiable, the slices were scanned using a Zeiss Axio Imager M2 microscope. Next, the pyramidal layer of CA1 was delineated using the AxioVison software (Zeiss), where pyramidal neurons were manually counted.

Proteinopathies

Every case was examined for beta-amyloid pathology, neurofibrillary tangles, Lewy bodies, FUS inclusions and TDP-43 pathology with a specific focus on the hippocampal regions.

The proteinopathy causative for the clinical syndrome was identified. The National Institute on Aging-Alzheimer’s Association Criteria were used for AD neuropathology diagnosis used (48); this was based on a Braak score for hyperphosphorylated tau pathology (49), Thal stages for amyloid

pathology (50), and CERAD staging of neuritic plaques (51). To assess the likelihood of clinical correlation, we referred to the NIA-Reagan Criteria (52).

Cases were divided into 4 groups depending on the likelihood for clinical correlation: the 4 groups of "Not", "Low", "Intermediate" or "High" correlation with clinical phenotype were evaluated based on the presence of HS. Lewy body pathology classification of McKeith et al was used (53), whereas we applied the Mackenzie criteria for TDP-43 proteinopathies (24).

Cerebrovascular Pathology and Cerebral Amyloid Angiopathy

Staging of cerebrovascular pathology was based on Deramecourt et al (54). Arteriosclerotic changes, perivascular hemosiderin deposits and dilation of the perivascular space together with hemorrhages and micro or lacunar infarcts were representative of CVD. Three groups were created: no or mild vascular alterations (score 0 or 1 of Deramecourt); moderate alterations (=score 2 of Deramecourt) or severe vascular changes (score 3 or 4 of Deramecourt), hemorrhages or infarctions. As infarctions are end-stage evidence of cerebrovascular pathology, infarctions in basal ganglia were also separately analyzed.

Cerebral amyloid angiopathy (CAA) pathology was scored as suggested by Love et al as follows: 0: no CAA; 1: sporadic (trace of occasional vessel affected); 2: moderate (one of a few vessels circumferentially affected); and 3: severe (widespread involvement of circumferentially affected vessels) (55). Cerebrovascular pathology was independently rated by 2 assessors blinded from each other (MP and AS) (Cohen's K: 0.527).

Age

Age of death was included as a continuous variable. However, as many studies include >80 years old as a specific advanced age group, this additional variable was also created.

Statistical Analysis

The presence of HS was the primary outcome measure in this study. The discriminating ability of the quantitative neuronal cell count to predict HS was investigated through ROC curve analysis (AUC). Mean neuronal cell count in the HS group versus the non-HS group was evaluated using an independent t-test.

Exploratory data analysis was performed by reporting the number of HS cases per level of categorical variables including: (i) FTLD-TDP, (ii) FTLD- TPD subtype, (iii) TDP-43 in hippocampus, (iv) p62 pathology in hippocampus, (v) advanced age (>80), (vi) Thal score, (vii) Braak score, (viii) AD neuropathological changes based on likelihood for clinical correlation, (ix) severity of vascular

pathology in the deep white matter and basal ganglia, (x) infarctions in deep white matter and basal ganglia, (xi) cerebral amyloid angiopathy, (xii) age as a continuous variable, and (xiii) Deramecourt total score.

Simple logistic regression analyses were performed to investigate the influence of different variables on outcome measure. In the multivariable logistic regression analysis, associations between relevant variables and outcome measures were examined. Likelihood ratio tests were used due to small sample sizes. Estimated odds ratios and their 95% profile likelihood confidence intervals were reported. P values <0.05 were considered significant. All statistical analyses were performed in SPSS 25 (IBM SPSS version 25 Software, IBM, Corp., Armonk, NY).

RESULTS

Description of Study Population

Our final study cohort consisted of 122 cases, of whom 73 showed FTLD or FTLD-MND pathology (68 FTLD and 5 FTLD-MND cases), whereas 49 cases had a non-FTLD-MND neuropathology diagnosis: 28 cases with AD as primary neuropathological diagnosis, 5 cases with severe cerebral vascular pathology (VAD), 8 patients with MND (without FTLD) and 8 controls without major neuropathological findings (Table 1). Gender differences were evenly distributed over neuropathological subgroups. Mean age of death was 66.9 years (from 34 to 92 years old), 14 cases were classified as advanced age (>80 years old) (Table 1)

Neuropathology

FTLD Proteinopathies

Of the 73 FTLD subjects, 66% had FTLD-TDP, with FTLD-TDP type B being the most prevalent (41%). The second most common subtype was FTLD-TDP type A (16%), followed by FTLD-TDP type D pathology (6%). Only 1 subject had FTLD-TDP type C. The 5 subjects with FTLD-MND-TDP had TDP-43 type B pathology. The remaining FTLD population was either diagnosed with a tauopathy: PSP/CBD tauopathy (20.5%), Pick disease (5.5%), GGT (2.7%) and AGD (1 case), or with FTLD-FUS (2 cases), or with FTLD-UPS (1 case) (Fig. 1A-C). In the non-FTLD counterparts, 7 out of 8 MND subjects had a TDP-43 proteinopathy, while 1 subject had MND pathology related to *SOD1* mutation with ubiquitinated inclusions in the motor neuron cells.

Hippocampal Proteinopathies

Hippocampal TDP-43 pathology was found in 60.0% of all cases. Of these cases with hippocampal TDP-43, 60% were associated with FTLD-TDP (27.9% FTLD-TDP A, 60.5% FTLD-TDP B, 2.3% FTLD-TDP C, 9.3% FTLD-TDP D). In the remaining 40% of hippocampal TDP-43 cases without associated FTLD-TDP, AD was present in 48%, whereas 31% had PSP/CBD. FTLD-UPS, GGT, VAD, Pick, MND-TDP and MND-Ubi were present in 3.4% (Tables 1 and 2). None of the control cases had any hippocampal TDP-43. In 5 cases, mesotemporal TDP-43 pathology was suggestive for LATE. In the hippocampal TDP-43-negative group (48 of 120 cases), 6% (n = 3) had p62-immunoreactive, tau-negative and α-synuclein-negative neuronal cytoplasmic inclusions in the hippocampus.

Vascular Changes

On the whole, 28% of cases had none to mild CVD in the basal ganglia and deep white matter, whereas 47.4% had moderate CVD, and 25.0% had severe CVD. Focusing on the presence of

infarctions as the end stage of CVD, we found infarctions in the deep white matter and basal ganglia in 16.4% of cases (n = 19). Infarctions that were spread over the entire brain were found in 24.1% of cases (n = 28). Mean Deramecourt total score was 5.4 out of 20 (SD 2.6). Minimum score was 0 out of 20, and maximum score was 14 out of 20. Six cases were excluded from analysis, as there was no possibility to score thalamus and/or basal ganglia.

Cerebral Amyloid Angiopathy

8.8% (n = 10) had sporadic CAA changes, 11.4% (n = 13) had circumferential CAA pathology, 14% (n = 16) had widespread CAA. 65.8% (n = 75) of the patient population did not have any CAA. Twelve cases were excluded due to technical factors, such as absence of sampling.

Alzheimer Disease

In total, 71 cases were identified with AD pathology: a high correlation (see [52]) with clinical phenotype was present in 14.8% ($A_3B_3C_{2/3}$), whereas 12.3% had intermediate AD pathology ($A_{1/2}B_{2/3}C_{2/3}$ to $A_3B_2C_{2/3}$) and in 31.1% of cases, the AD pathology was considered to be low ($A_{1/2}B_{1/2/3}C_1$ to $A_{2/3}B_1C_{1/2/3}$). In the 28 cases that had AD as a primary diagnosis, 18 had a high correlation with clinical phenotype (i.e. severe AD pathology), 9 had intermediate AD pathology and 1 subject had only mild AD pathology. In 43 cases, AD pathology occurred as coexisting pathology (i.e. considered not to be a factor contributing to the clinical phenotype) including in 4 out of 8 clinically healthy controls. In most cases, AD pathology was considered to be low.

Hippocampal Sclerosis

In total, 24 out of 122 patients had HS (Fig. 2). Out of these 24 cases, 18 HS subjects had FTLD(-MND), of which FTLD-(MND)-TDP was present in 13 of HS cases. Three HS subjects had FTLD-tau, 2 HS subjects had FTLD-FUS and 6 HS subjects had AD (Table 2).

Association Between Proteinopathies, Vascular Disease, Age and HS

There was no association between FTLD-(MND)-TDP and HS ($p = 0.101$). Six out of 12 subjects with FTLD-TDP A had HS, whereas only 2 out of 30 subjects with FTLD-TDP B had HS. HS was not present in 1 FTLD-TDP C case, but all 5 subjects with FTLD-TDP D had HS ($p < 0.001$) (Table 3). In the FTLD-Tau group, no associations could be made between the tauopathies and HS. The 2 subjects with FTLD-FUS had HS. In the MND, VAD and control groups, no HS was present. (Table 1). In the 28 subjects with AD, 6 had HS (21.4%). Addition of the AD pathology to the regression model did not improve the model nor the prediction of the presence of HS ($p = 0.902$).

There was a strong association between hippocampal TDP-43 pathology and HS (p = 0.002), regardless of neuropathological primary diagnosis, with hippocampal TDP-43 present in 20 of 24 HS cases (Tables 2 and 3). Additionally, p62-positive, TDP-43-negative hippocampal inclusions were not associated with HS (p = 0.246).

Vascular changes in deep white matter and basal ganglia were analyzed in 116 cases. There was an association between severe vascular changes and HS presence in 9 out of 29 cases (p = 0.034). In the group with no to mild vascular changes, 2 out of 32 subjects had HS (6.3%), whereas 11 out of 55 subjects with moderate vascular changes (20.0%) had HS, and 9 out of 29 subjects with severe vascular changes had HS (31.0%).

There was no association between infarctions at the level of deep white matter and basal ganglia and thalamus, and HS (p = 0.694). The Deramecourt total score in the “no HS” group was 5.48 (standard deviation of 2.748) versus 5.14 (standard deviation of 1.957) in the “HS” group, showing no value as a predictor of HS (p = 0.591) (Table 3). Cerebral amyloid angiopathy was present in 39 of 114 cases. Sixteen of 39 demonstrated severe CAA, of which 3 had HS (p = 0.156).

In our study population, the mean age in the “no HS” group was 67.16 (10.218) and in the “HS” group was 65.58 (12.796), showing no association between HS and age (p = 0.518). In the 14 cases over >80 years, 3 had HS (p = 0.862). We could not find any significant predictive value of advanced age (>80), hippocampal TDP-43, severe vascular pathology or CAA.

Because hippocampal TDP-43, FTLD-TDP type D and severe vascular pathology in deep white matter tracts were considered significant predictor variables for HS in this univariable logistic regression analysis model, we consequently examined these variables following multiple variable regression analysis. However, after inclusion of these variables, only hippocampal TDP-43 and FTLD-TDP subtype D remained significant predictive factors for HS (Table 4).

Neuronal Count in CA1

The visual score was compared to the absolute neuronal number count in CA1. A total of 115 out of 122 cases were completed. As expected, the mean neuronal number in CA1 was higher in the group without hippocampal sclerosis than in the group with neuronal loss in CA1. The mean pyramidal neuronal cell count in CA1 in the “no HS” group was 586.11 on average (95% CI: 537.59; 634.63), whereas in the “HS” group, the mean number was 249.77 (95% CI: 177.09; 322.46) (Fig. 3A).

With these results, it could be assumed that our semiquantitative visual rating scale was sufficient to discriminate whether HS was present or not. This was also illustrated with an AUC (area under the

curve) value of 0.90 (95% CI: 0.83; 0.97), thus assuming that neuronal counting in CA1 has sufficient discriminatory power to identify the presence of HS (Fig. 3B; sensitivity: 82%, specificity: 82%, Youden Index: 0.635).

DISCUSSION

In this autopsy cohort of mostly early-onset dementia patients, we confirmed the strong association between HS and hippocampal TDP-43, in addition to that with FTLD-TDP type D, and to a lesser extent with FTLD-TDP type A. Nearly all of the subjects with FTLD-TDP had TDP-43 pathology in the hippocampus (Table 2), while 6 out of 12 in the FTLD-TDP A cohort, 2 out of 30 in the FTLD-TDP B cohort and all subjects in the FTLD-TDP D cohort had HS (n = 5). Interestingly, our data also showed an association between HS and severe vascular changes in the deeper white matter and basal ganglia.

Hippocampal TDP-43 Pathology and HS

The specific concept of "Pathoklise" was proposed by Vogt and Vogt, suggesting that intrinsic hippocampal neuronal cell characteristics lead to the susceptibility of cell death (56). In 1986, Rothman and Olney showed that the neurons of CA1 have more glutamate receptors compared to the other more resilient hippocampal neurons. Disease states such as epilepsy have been known to induce an overabundance of glutamate in the synapse, which can be toxic due to postsynaptic Ca^{2+} influx. Neurons that are richer in glutamate receptors are more prone to cell death, referred to as glutamate excitotoxicity (57). Additionally, the more vulnerable CA1 neurons have less Ca^{2+} buffering capacity, due to small concentrations or even absence of calbindin and chromogranin A compared to more resistant neurons (58). Therefore, a poor ability to protect against the excitotoxicity effect of glutamate and Ca^{2+} influx, is the pathogenetic mechanism of HS in conditions such as epilepsy.

In neurodegeneration, the pathogenesis of HS is not completely understood. The link between glutamate excitotoxicity and neurodegeneration is not clear. Other pathways have been suggested to have an impact on neuronal loss in CA1, such as an altered inhibitory input (59). Different groups also hypothesized that CA1 neurons have an impaired degradation of proteins, thus leading to an accumulation of possible toxic protein deposits (60-62).

The association between TDP-43 pathology and neuronal loss has been explained by a pathophysiological mechanism through reactive oxygen species (ROS) and a deficient or dysfunctional heat shock response. This leads to an accumulation of TDP-43 in stress granules and the subsequent formation of toxic TDP-43 oligomers (63, 64). We suggest that this mechanism occurs in every cell affected by TDP-43 inclusions, and depending on intrinsic cell qualities, neurodegeneration and sclerosis occur, as seen in CA1.

A relation between hippocampal TDP-43 pathology and HS in epilepsy has not been found, nor a relation between FTLD and epilepsy (65, 66), but recent data in the epilepsy research field suggest a cognitive decline in patients with TLE, with tau pathology being present in TLE-associated HS cases. Also, alpha-synuclein has been reported to be higher in cerebrospinal fluid and serum of patients with refractory epilepsy (67, 68). In our study, we did not find any relation between HS and hippocampal tauopathy nor synucleinopathy. Furthermore, when looking at the severity of the AD pathology, we could not find any association with HS. No link between HS and cerebral amyloid angiopathy was found.

HS and Cerebrovascular Pathology

The significant association between HS and cerebrovascular pathology, suggests a vascular contribution to the development of HS. This was previously hypothesized by Spielmeyer (69) and Uchimura (quoted by Boling et al [70]) and recently reproduced by Neltner et al and Zarow et al (41, 43). Spielmeyer stated that CA1, being most compromised in HS, has a suboptimal vessel supply compared to neighboring parts, as it is located in a watershed area between the anterior choroidal artery and posterior cerebral arteries (71). Recently, the differences between the hippocampal and neocortical vasculature, with a challenging small number of capillary anastomoses in the hippocampus and high degree of interindividual variations of hippocampal blood supply, have also been proposed as a potential underlying predisposing factor following 7-Tesla MRI studies (72, 73). Next to local ischemic events, other groups have suggested a systemic hypoxia/ischemia hypothesis, while still others have assumed a disruption of the blood-brain barrier (74-76).

In 2012, a staging system was developed to rate cerebrovascular changes, which allowed standardizing and semi-quantifying cerebral vascular changes (54). This staging scheme was used to score cerebrovascular pathology in frontal and temporal lobes, as well as deep white matter at the level of the thalamus and basal ganglia. Because previous studies described a correlation between arteriolosclerosis and HS (41), between deep white matter hyperintensities (WMH) and hippocampal volume (40), and between deep WMH and hippocampal regional cerebral blood flow (77), we focused on the cerebrovascular pathology in basal ganglia and deep white matter.

Using this staging system, we did not find any association between the total vascular score and HS, but we did find a relation between HS and the severe cerebrovascular alterations in deeper white matter tracts and basal ganglia, i.e. arteriolosclerosis, perivascular demyelination, and perivascular hemosiderin deposition. These findings are in line with the findings of Neltner et al (41).

In ischemic conditions, CA1 neurons are more vulnerable compared to other neurons, a phenomenon which has been explained by the “calpain-cathepsin” hypothesis. In CA1 neurons, the presence of ROS and altered Ca^{2+} homeostasis, as a result of hypoxia or ischemia, leads to the carbonylation of heat shock protein 70 (HSP70), inducing a destabilization of the lysosome. The carbonylated HSP70 is cleaved by calpain, which causes a loss of integrity of the lysosomal membrane. Due to leakage of the lysosomal membrane, cathepsin spills into the cell cytoplasm, which in turn induces the degeneration of CA1 neurons. In other neurons this calpain-cathepsin axis does not exist, probably because of less mobilization of intracellular calcium, compared to CA1 neurons (75, 78).

Cerebrovascular Pathology and TDP-43

In 2007, Lee et al (66) did not find any TDP-43 pathology in anoxic and ischemic lesions. In contrast to these findings, Katsumata et al (44) found an association between arteriolosclerosis pathology and TDP-43 in the amygdala and entorhinal cortex in persons without *APOE* Ɛ4 homozygosity. Additionally, in 2010, Geser et al found that certain chronic vascular diseases have the potential to drive the phosphorylation and misfolding of TDP-43 (79). However, further data exploring possible pathways between TDP-43 and vascular pathology are lacking, and also the design of our study does not allow any statements about this possible interaction.

Advanced Age

Whereas different studies and reviews have been published emphasizing the association between HS and advanced age (42, 80), we could not find any association between these 2 variables. However, the difference between our study and previously published reports is that the mean age in our group was 66.9 years, whereas, to our knowledge, the average age in other studies was higher. As the focus of our study was on early-onset dementia patients, we can assume that our group of elderly patients was too small to draw conclusions about any relation between advanced age and HS. Even if we consider the relatively long disease course and/or advanced age of some cases, there was no evident association with HS, unlike other publications (18). Nevertheless, based on our results, the presence of HS also needs to be considered in early-onset dementias.

Neuronal Count in CA1

Additionally, we were able to associate the semiquantitative visual diagnosis of HS with a quantitative neuronal cell count in CA1. This implies that the neuronal cell loss is sufficient for diagnosing HS. If future research identifies pyramidal neurons in CA1 and the subiculum, we suggest that quantitative measurement is preferential in the diagnosis of hippocampal sclerosis.

Limitations

Our study has several limitations. We were not able to make any conclusion concerning symmetrical or asymmetrical hippocampal involvement, as our research protocol includes that only the right hemisphere is processed for neuropathological analysis, whereas the left is stored at -80°C for further genetic or neurochemical analyses. Furthermore, examining the right brain hemisphere could be a cause of underdiagnosing HS, as TDP-43 pathology mostly occurs bilaterally in the hippocampus, whereas one should keep in mind that in 40% to 55% of cases, HS is only asymmetrical, inducing a false negative detection of HS in approximately 25% of HS cases (11, 43). This could induce an underestimation of the correlation between TDP-43 and HS. Overall, the small number of cases in our study makes our statistical analyses tenuous. However, it allows us to detect the tendency that HS is strongly associated with TDP-43 pathology in the hippocampus, and we assume that small vessel changes play an additional role, as previously detected in older cohorts (41). Our study lacked a systematic genetic analysis, due to the retrospective concept of our study.

Conclusions

We were able to confirm the strong relationship between hippocampal TDP-43 pathology and HS. We found that the FTLD-TDP type D and to a lesser extent type A cases more frequently showed HS. Interestingly, the odds for HS were significantly higher when subjects had more severe vascular changes in deeper white matter and basal ganglia.

Due to the concept of this autopsy study, with HS as endpoint, we cannot make a statement about any relation between cerebrovascular disease and TDP-43 pathology. Referring to our data, we assume that cerebrovascular disease does not have a direct impact on development of HS. Possible mechanisms of action could include a double hit phenomenon with hippocampal TDP-43 pathology and cerebrovascular pathology leading to HS, or cerebrovascular pathology acting as a modulatory contributing factor on TDP-pathology and thus HS. An upstream effect inducing cerebrovascular pathology, TDP-43 pathology and HS separately from each other, can neither be ruled out.

Furthermore, a quantitative neuronal cell count in CA1 was introduced to objectify the semiquantitative visual appreciation of HS, implying that neuronal cell loss in CA1 is sufficient for diagnosing HS. We propose that quantitative measurement is preferential in the diagnosis of hippocampal sclerosis.

We suggest that research should also focus on the assessment of hippocampal functioning in the workup of FTD patients, through imaging and neuropsychological testing, as this could be a biomarker of TDP-43 pathology and vascular risk factors. Additionally, the systematic gene analysis

with screening of the known FTLD-TDP associated genes – but also of the HS risk genes – may be the next step forward in identifying and clarifying dementia-specific pathophysiological mechanisms of TDP-43 leading to HS.

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Legends to

Figure 1. (**A**) Pie chart with percentages of proteinopathy associated with clinical syndrome. (**B**) Pie chart demonstrating percentages of FTLD-TDP subtypes. (**C**) Pie chart with percentages of FTLD-tau subtypes. FTLD: frontotemporal lobar degeneration; FTLD-tau: frontotemporal lobar degeneration with tauopathy; FTLD other : collection of all non-tau-non-TDP FTLD cases; FTLD-TDP: frontotemporal lobar degeneration with TDP-43 proteinopathy; MND: (pure) motor neuron disease without FTLD; AD: Alzheimer disease; VAD: vascular disease; mixed: mixed dementia VAD + AD. AGD: argyrophilic grain disease; CBD: corticobasal degeneration; GGT: globular glial tauopathy; PSP: progressive supranuclear palsy

Figure 2. Hippocampal sclerosis with neuronal loss in CA1 demonstrated by CV stain. (**A**) AD. (**B**) FTLD-tau (Pick disease). (**C**) FTLD-FUS. (**D**) FTLD-TDP type A. (**E**) FTLD- TDP type B. (**F**) FTLD-TDP type D. Note the relatively mild neuronal loss in CA1 in **E** (*CA1).

Figure 3. (**A**) Box plots show the mean cell count in CA1 in the group without HS ('no') versus the group with HS ('HS'). As expected, the mean neuronal number in CA1 is lower in the 'HS' group. (**B**) ROC curve with AUC of cell count in CA1 plotted against HS.

Figure

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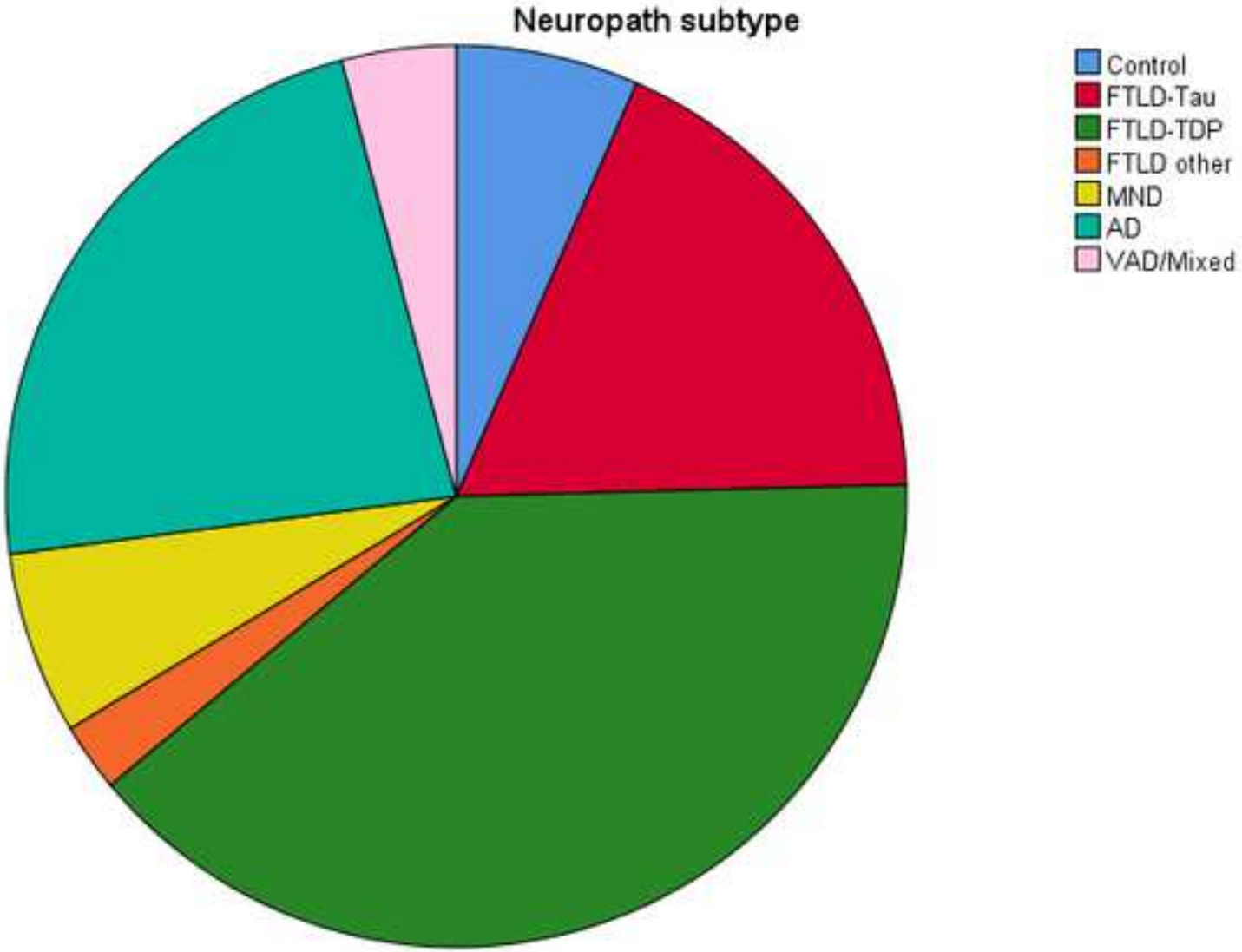

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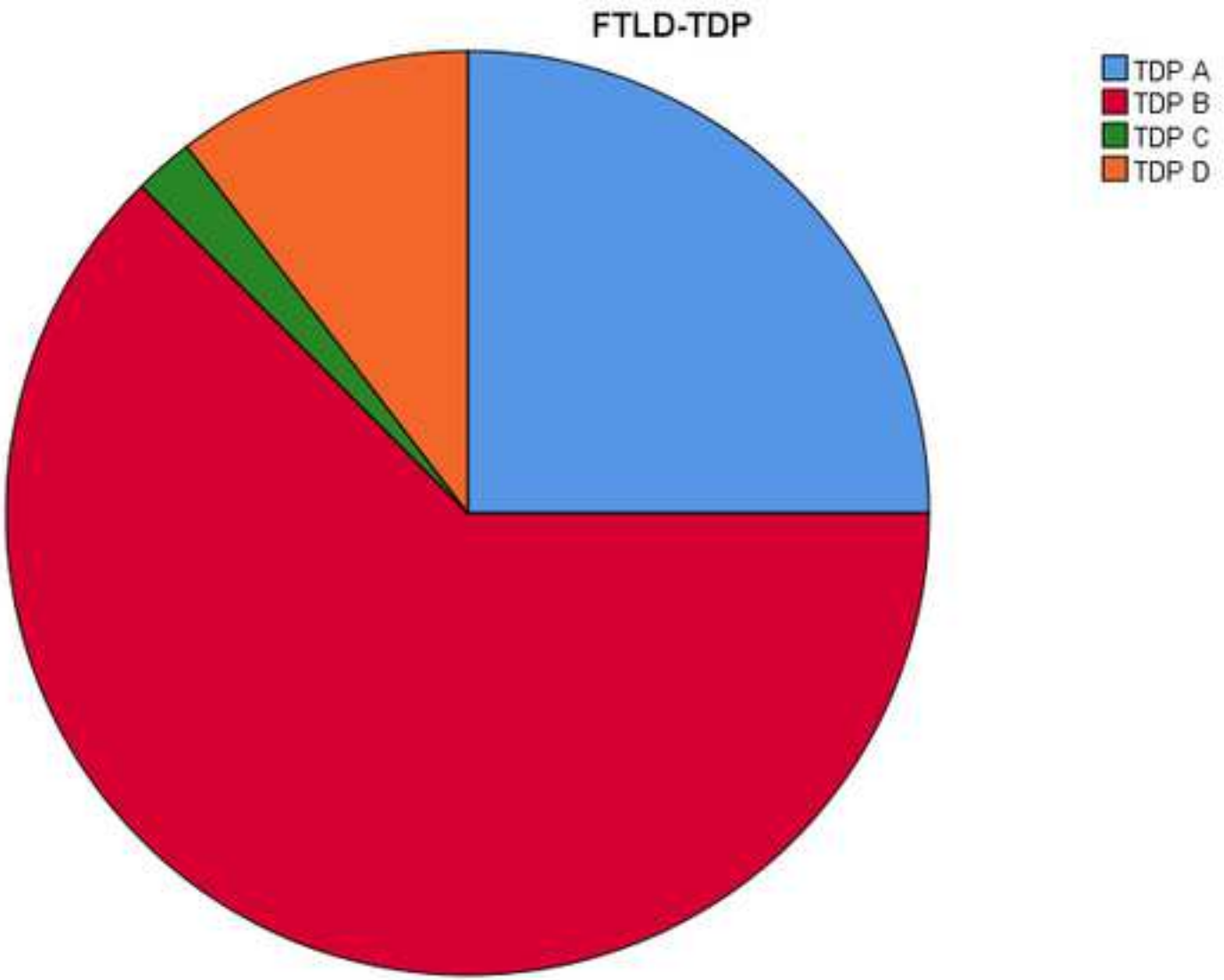

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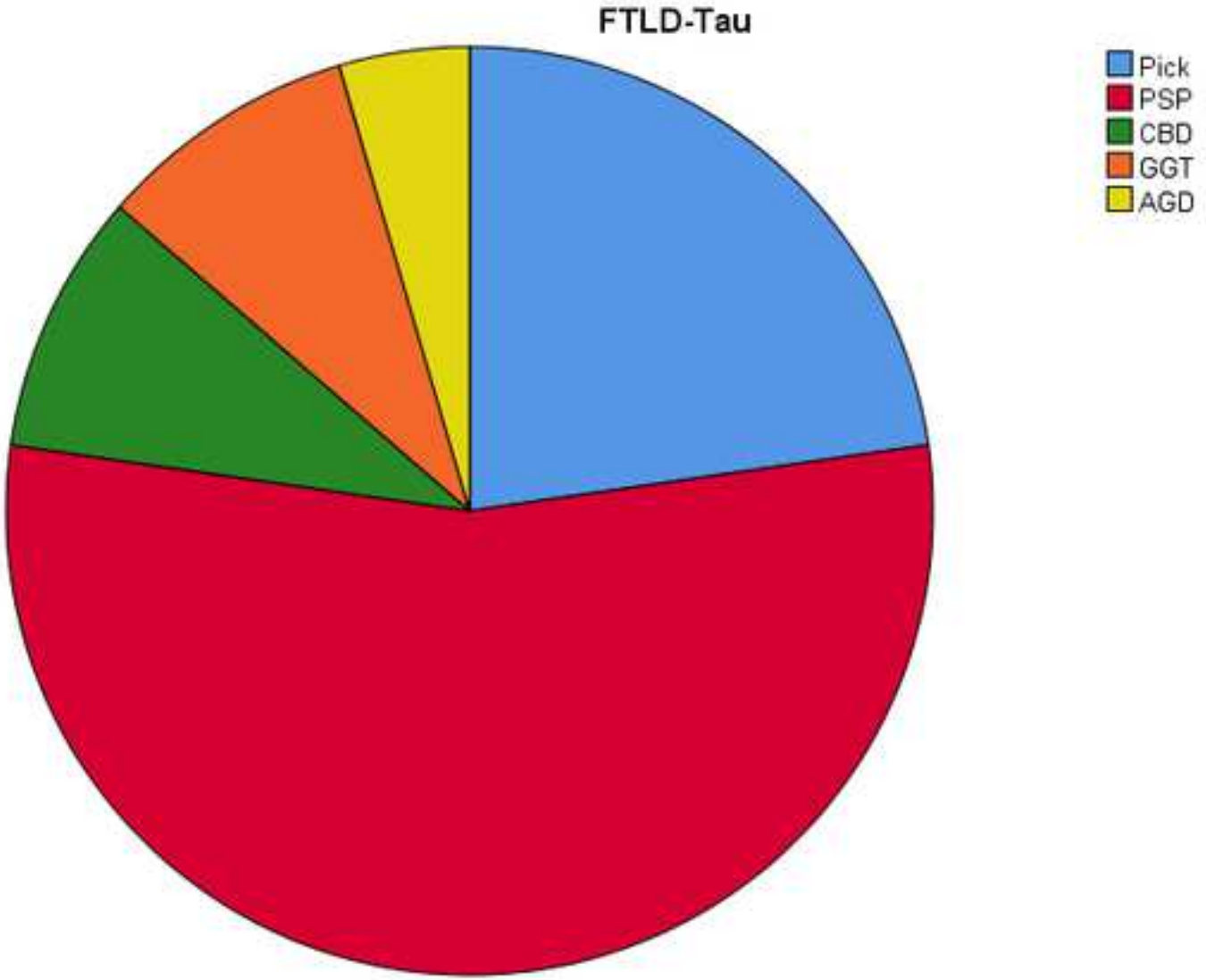

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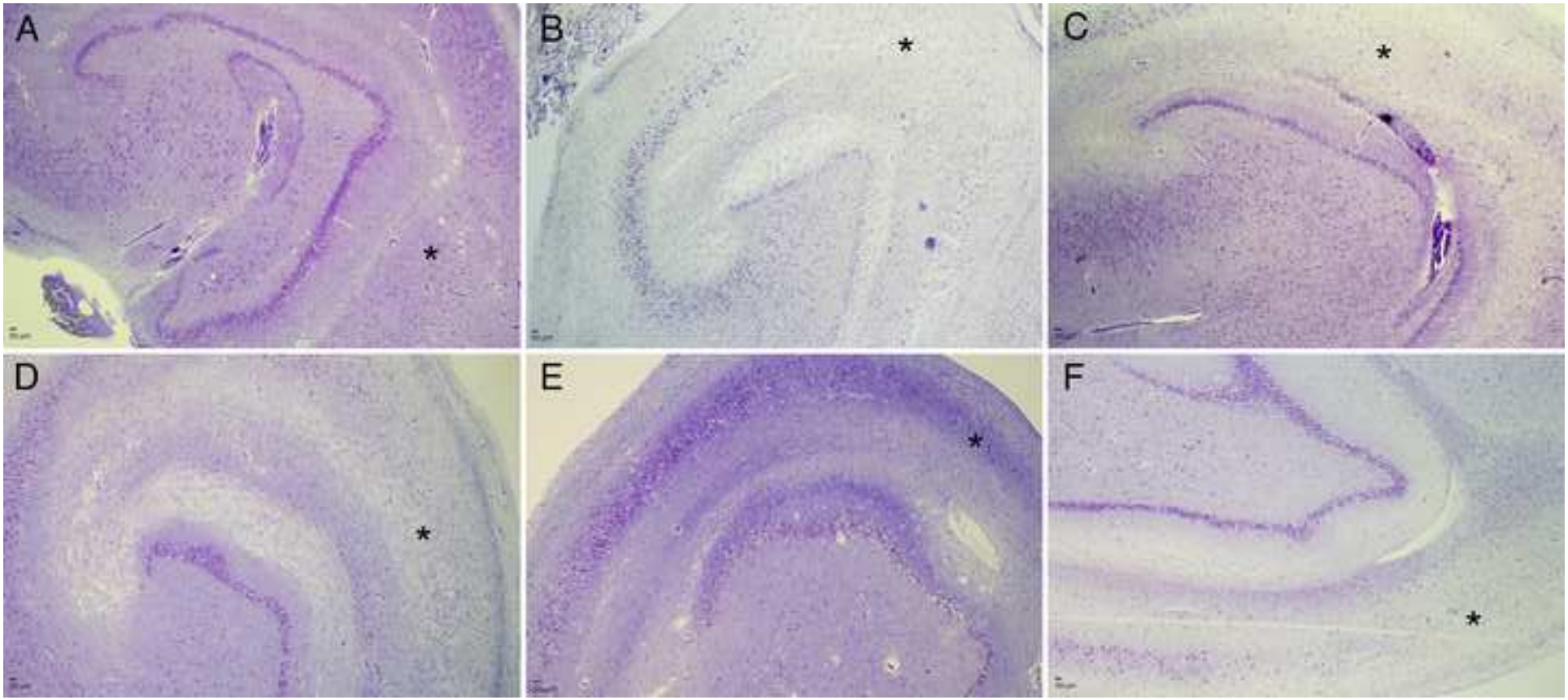

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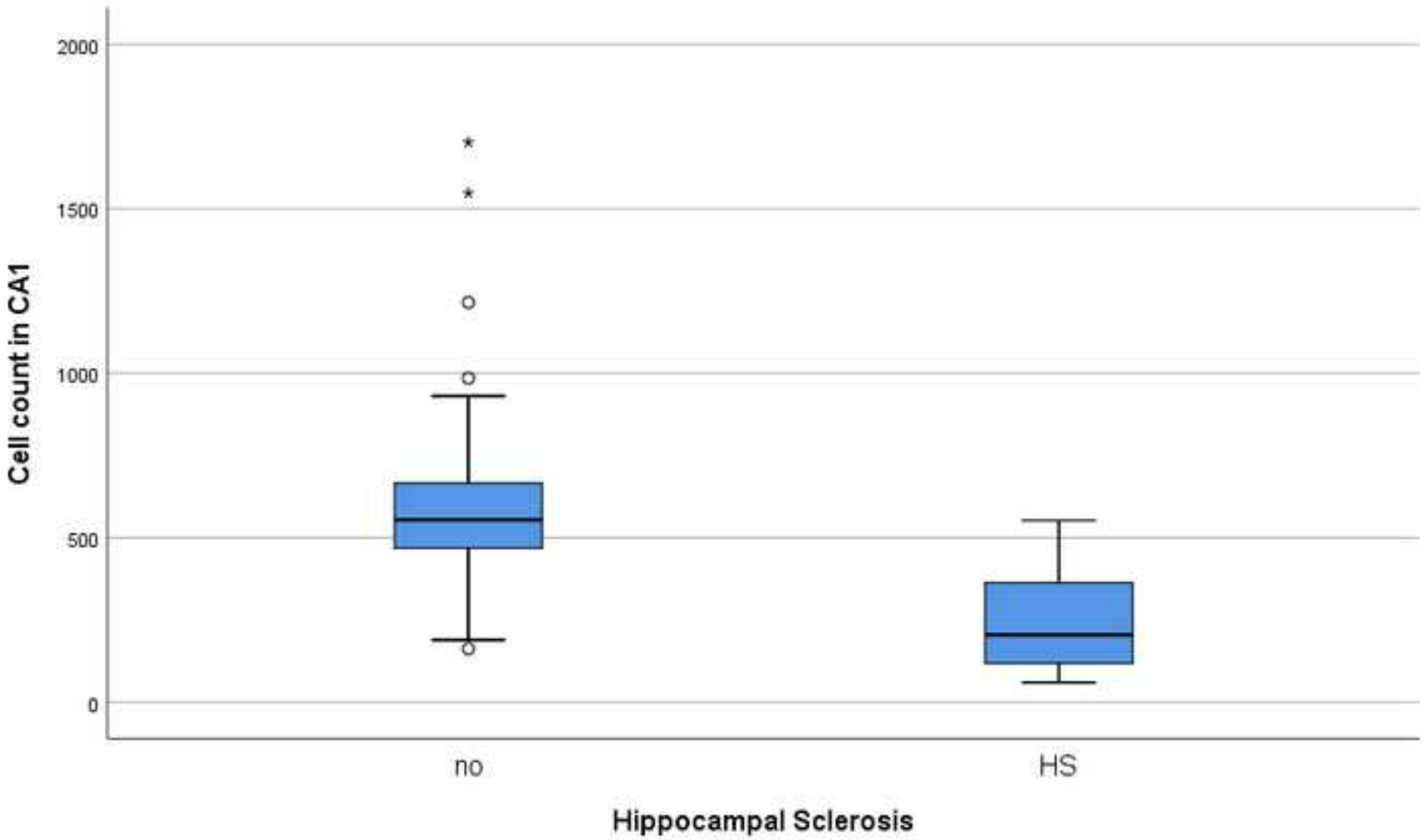

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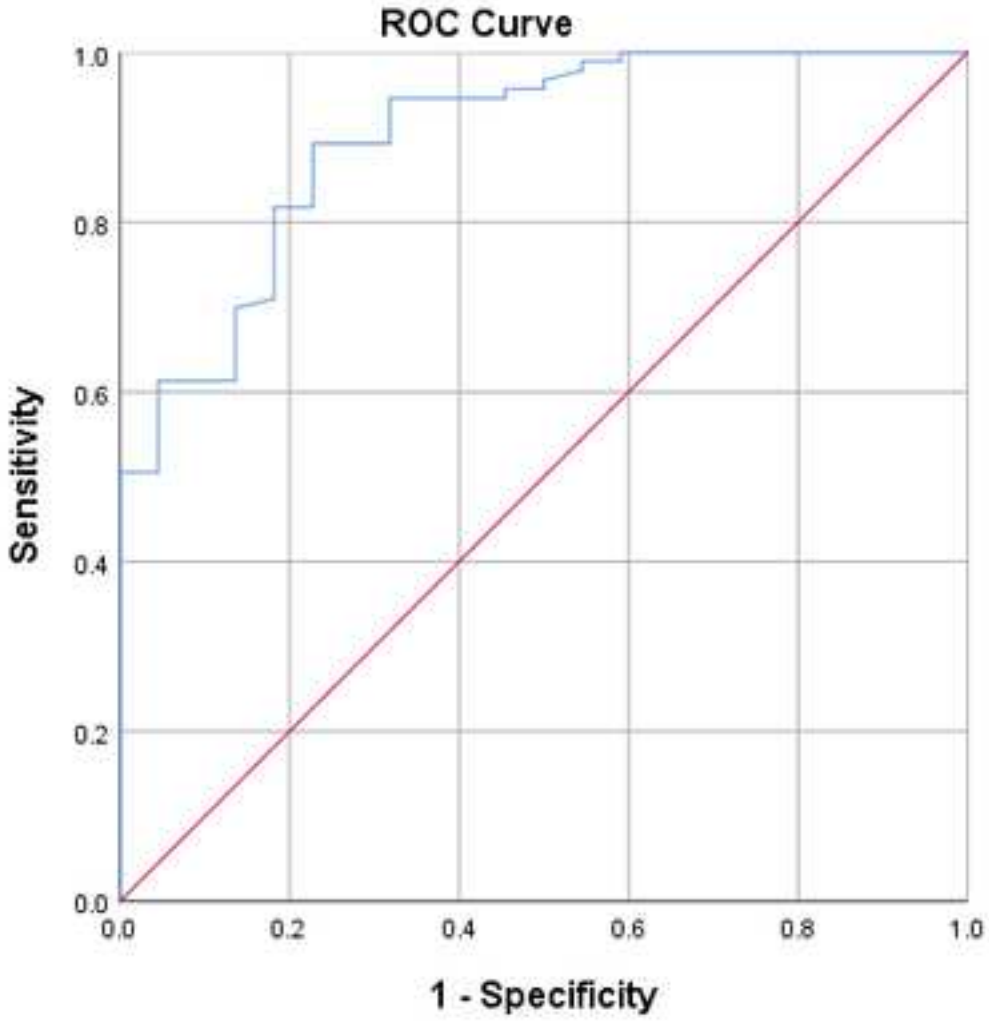

Table

Table 1. Study Population

Neuropathological diagnosis	Number of patients (%)	Gender (F/M)	Mean Age of death (SD)	HS (% of row)	Hippocampal TDP-43 (%)	Vascular pathology in Basal Ganglia (N/M/S%)	Deramecourt Total score Mean (SD)
Control	8 (6.6)	5/3	73.3 (8.05)	0 (0)	0/8 (0)	12.5/62.5/25	6.5 (3.55)
FTLD	68 (55.7)	29/39	66.3 (10.13)	17 (25)	53/66 (80.3)	18.5/49.2/32.3	5.5 (2.39)
FTLD-MND	5 (4.1)	4/1	60.0 (8.63)	1 (20)	2/5(40)	60/20/20	2.8 (2.59)
MND	8 (6.6)	2/6	59.0 (8.63)	0 (0)	2/8 (25)	50/25/25	3.7 (1.67)
AD	28 (23.0)	16/12	69.6 (10.40)	6 (21.4)	14/28 (50)	44/44/12	5.8 (2.74)
VAD	5 (4.1)	1/4	68.6 (14.78)	0 (0)	1/5 (20)	20/80/0	6.0 (2.92)
Total	**122 (100%)**	**57/65**	**66.9 (10.73)**	**24 (19.7)**	**72/120 (60.0)**	**28/47/25**	**5.4 (2.61)**

Percentages are per neuropathological diagnosis. Vascular pathology is divided into 3 groups: 1 group without any or mild vascular alterations (N – normal), 1 group with moderate alterations (M - moderate) and 1 group with severe vascular changes or with hemorrhages or infarctions (S – severe). Total Deramecourt score varies between 0 and 20 max.

HS: hippocampal sclerosis ; Hippocampal TDP-43: hippocampal TDP-43 proteinopathy ; FTLD: frontotemporal lobar degeneration ; FTLD-MND: frontotemporal lobar degeneration with motor neuron disease ; MND: (pure) motor neuron disease without FTLD ; AD: Alzheimer disease ; VAD: vascular disease.

Table

Table 2. Hippocampal TDP Pathology in Relation to Neuropathological Diagnosis and ~~Presence of~~ HS

Neuropathological diagnosis	Number of patients	Cases with Hipp TDP		Cases without Hipp TDP	
		Total	Cases with HS	Total	Cases with HS
FTLD-tau	**22**	**11**	**2**	**11**	**1**
AGD	1	0	-	1	0
CBD	2	2	1	0	-
GGT	2	1	0	1	0
Pick	4	1	1	3	1
PSP	13	7	0	6	0
FTLD-(MND)-TDP	**46**	**43**	**12**	**3**	**0**
TDP A	12	12	6	0	-
TDP B*	29	26	2	3	0
TDP C	1	1	0	0	-
TDP D	4	4	4**	0	-
FTLD-FUS	**2**	**0**	**-**	**2**	**2**
FTLD-UPS	**1**	**1**	**0**	**0**	**-**
Control	**8**	**0**	**-**	**8**	**0**
MND	**8**	**2**	**0**	**6**	**0**
MND-TDP	7	1	0	6	0
MND-ubi	1	1	0	0	0
AD	**28**	**14**	**6**	**14**	**0**
VAD	**5**	**1**	**0**	**4**	**0**
Total	**120 (2 missing)**	**72**	**20*** **	**48**	**3**

Numbers are absolute numbers. ~~In this table o~~Only 71 FTLD cases are included because~~, as~~ hippocampal TDP43 assessment was not possible in 2 cases.
*Five cases with FTLD-MND-TDP had TDP type B pathology
****Five FTLD-TDP cases had HS; hippocampal TDP pathology could be assessed in only 4 cases.
*****Absolute number of HS cases is 2~~0~~3 + 3 = 23, as a result of 1 case (see *) that could not be assessed.

HS: hippocampal sclerosis; Hipp TDP: hippocampal TDP-43 proteinopathy; FTLD: frontotemporal lobar degeneration; FTLD-tau: frontotemporal lobar degeration with tauopathy; AGD: argyrophilic grain disease; CBD: corticobasal degeneration; GGT: globular glial tauopathy; PSP: progressive supranuclear palsy; FTLD-(MND)-TDP: frontotemporal lobar degeneration (with motor neuron disease) with TDP-43 proteinopathy; FTLD-FUS: frontotemporal lobar degeneration with fused-in-sarcoma proteinopathy; FTLD-UPS: frontotemporal lobar degeneration with ubiquitin immunoreactive inclusions; MND: (pure) motor neuron disease without FTLD; MND-TDP: motor neuron disease with TDP-43 proteinopathy; MND-ubi: motor neuron disease with ubiquitin immunoreactive inclusions; AD: Alzheimer disease; VAD: vascular disease.

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Table

Table 3. Univariate Logistic Regression Analysis

Variables		HS (%)	Univariable logistic regression analysis		
			Unadjusted OR	95%CI	p-value
FTLD-(MND)-TDP	Yes	13/48 (27.1)	2.13	0.86-5.34	0.101
	No	11/74 (14.9)			
TDP-43 subtype	TDP-43 A	6/12 (50)	4.91	1.38-17.81	**<0.001 (0.0001)**
	TDP-43 B	2/30 (6.7)	0.43	0.08-1.60	
	TDP-43 C (*)	0/1 (0)			
	TDP-43 D	5/5 (100)	54.05	5.52-7280.83	
	MND	0/7 (0)	0.33	0.00-3.04	
	No TDP-43	11/67 (16.4)			
Hippocampal TDP-43 (2 missings)	Yes	20/72 (27.8)	5.77	1.83-25.62	**0.002**
	No	3/48 (6.3)			
Hippocampal P62	Yes	2/19 (10.5)	0.43	0.07-1.67	0.246
	No	22/103 (21.4)			
Advanced age of death	>80	3/14 (21.4)	1.13	0.24-4.01	0.862
	<80	21/108 (19.4)			
AD neuropathological changes	high	3/18 (16.7)	0.82	0.17-3.12	0.902
	moderate	4/15 (26.7)	1.49	0.36-5.47	
	low	7/38 (18.4)	0.93	0.31-2.69	
	none	10/51 (19.6)			
Cerebrovascular changes in basal ganglia (6 missings)	Severe	9/29 (31)	6.75	1.54-47.41	**0.034**
	Moderate	11/55 (20)	3.75	0.92-25.36	
	None to Mild	2/32 (6.3)			
Infarction in basal ganglia (6 missings)	Yes	3/19 (15.8)	0.77	0.17-2.61	0.694
	N	19/97 (19.6)			
Cerebral amyloid angiopathy	3	3/16 (18.8)	1.01	0.23-4.04	0.156
	2	4/13 (30.8)	2.12	0.51-7.66	
	1	0/10 (0)	0.00	0.00-0.00	
	0	13/75 (17.3)			
		Mean (SD)			
Age of death	HS	65.6 (12.80)	0.99	0.95-10.3	0.518
	No HS	67.2 (10.22)			
Deramecourt total score	HS	5.1 (1.96)	0.95	0.78-1.14	0.591
	No HS	5.5 (2.75)			

(*) TDP-43 type C was excluded from statistical analysis due to small sample size (n = 1).

HS: hippocampal sclerosis; FTLD-(MND)-TDP: frontotemporal lobar degeneration (with motor neuron disease) with TDP-43 proteinopathy; Hippocampal TDP: hippocampal TDP-43 proteinopathy; Hippocampal p62: hippocampal p62 proteinopathy (TDP43 negative); MND: motor neuron disease; Montine: AD neuropathological changes cf NIA-AA criteria with cases grouped, based on high, moderate, low and no(ne) likelihood for clinical correlation; Deramecourt total score: score of cerebrovascular pathology (varies between 0 and 20 max).

Table

Table 4. Multivariable Logistic Regression Analysis

Multivariable logistic regression analysis				
		Adjusted OR	**95% CI**	**p-value**
Hippocampal TDP-43	YES	6.03	1.49-33.89	**0.011**
Cerebrovascular changes in basal ganglia	Moderate	2.97	0.59-28.96	0.202
	Severe	5.28	0.88-55.87	0.069
FTLD-(MND)-TDP (*)	TDP-43 A	1.48	0.34-6.38	0.597
	TDP-43 B	0.25	0.04-1.08	0.065
	TDP-43 D	23.88	1.77-3640.95	**0.014**
	MND	0.52	0.00-6.19	0.655
Age of death	>80	1.41	0.28-6.25	0.654

(*) TDP-43 type C was excluded from statistical analysis due to extreme small sample size (n=1).

HS: hippocampal sclerosis ; Hippocampal TDP-43: hippocampal TDP-43 proteinopathy ; FTLD-TDP: frontotemporal lobar degeneration with TDP-43 proteinopathy ; TDP-43 A ->D : subtypes of FTLD-TDP proteinopathy A->D respectively ; MND: motor neuron disease