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Hippocampal sclerosis in the elderly: genetic and pathologic findings, some mimicking Alzheimer disease clinically

Winnie C. Pao, M.D.[*], **Dennis W. Dickson, M.D.**[†], **Julia E. Crook, Ph.D.**[‡], **NiCole A. Finch, Ph.D.**[†], **Rosa Rademakers, Ph.D.**[†], and **Neill R. Graff-Radford, MBBCh, FRCP (London)**[*]

[*] Department of Neurology, Mayo Clinic, Jacksonville, FL

[†] Department of Neuroscience, Mayo Clinic, Jacksonville, FL

[‡] Department of Biostatistics, Mayo Clinic, Jacksonville, FL

Abstract

Background—Hippocampal sclerosis (HpScl) in the elderly is often associated with neurodegeneration.

Method—We studied the clinical and pathologic features of HpScl in 205 consecutive patients with dementia who came to autopsy from 1997 to 2008, focusing on associations with TDP-43 pathology and allelic variants in the progranulin (*GRN*) and apolipoprotein E (*APOE*).

Results—Of the 205 dementia patients, 28 had HpScl (14%). TDP-43 pathology was more frequent in cases with HpScl compared to those without HpScl (89% vs. 24%). *GRN* rs5848 T-allele but not *APOE* ε4 was associated with HpScl. In cases of HpScl with TDP-43 pathology and age of onset after 75 (n=11), 8 had AD-like amnestic syndrome, but most (6/8) had pathology not consistent with AD (Braak stage III or less), including 4 with frontotemporal lobar degeneration (FTLD-TDP), 1 with diffuse Lewy body disease and 1 with "pure HpScl."

Conclusions—HpScl is common in an elderly cohort with dementia, occurring in 14% of the cases in this series, and 89% have TDP-43 pathology, often associated with a risk variant in *GRN*. Patients with HpScl who present after age 75 often have presentations consistent with AD, but at autopsy have non-Alzheimer pathologies. Elderly patients with HpScl may be mistaken for AD.

INTRODUCTION

Hippocampal sclerosis (HpScl) is a pathological diagnosis characterized by selective neuronal loss and gliosis without cystic cavitation in the subiculum and hippocampal CA1 sector.[1] The reported prevalence of HpScl in the elderly is 3% to 13%.[2] While previously thought to be related to hypoxic/ischemic damage to the hippocampus,[3] more recently it has been associated with neurodegenerative processes.[2, 4, 5] It is found in over 75% of cases of frontotemporal lobar degeneration with TDP-43 inclusions (FTLD-TDP).[6] HpScl can be difficult to detect pathologically in the setting of Alzheimer's disease (AD) because of neurofibrillary degeneration in the same neuroanatomical distribution. It can only be diagnosed confidently when neuronal loss is disproportionate to the density of neurofibrillary tangles (NFTs), in particular the presence of extracellular NFTs.[1] In AD 31% of those with TDP-43 pathology have HpScl, whereas HpScl occurs in only 4% of AD cases negative for TDP-43.[2]

Correspondence: Dr. Neill R. Graff-Radford, Department of Neurology, Mayo Clinic, 4500 San Pablo Road, Jacksonville, FL 32224, Phone: 904-953-7103, graff-radford.neill@mayo.edu.

Progranulin (*GRN*) mutations cause FTLD-U with TDP-43 pathology.[7–9] The mutations produce null alleles, causing approximately 50% decrease in progranulin expression.[10] In addition to causative mutations, Rademakers et al. reported on a common variant in the 3′ untranslated region (3′UTR) of *GRN* (SNP rs5848), in a putative microRNA binding site, and found that the T-allele was associated with increased risk of FTLD-U and with decreased progranulin protein levels in the brain, probably due to translational repression mediated by the microRNA.[11] Analysis of two predominantly clinical FTLD case-control series, however, failed to identify a significant association with rs5848.[12, 13] Interestingly, a recent study explored the frequency of rs5848 in 644 autopsy-confirmed AD cases with respect to presence of HpScl (n=57) and TDP-43 pathology.[14] In that study the rs5848 T-allele was over-represented in AD with HpScl compared to those without HpScl. This finding suggested that a reduced progranulin level through rs5848 may be a risk factor for HpScl.

In a consecutive autopsy series of dementia patients followed prospectively at Mayo Clinic in Jacksonville, we describe the frequency, as well as the clinical and pathological features, of cases with HpScl, and their association with genetic variants in *GRN* and apolipoprotein E (*APOE).*

METHODS

Study subjects and clinical review

In the time period of 1997 to 2008, 221 patients who had been prospectively evaluated at Mayo Clinic in Jacksonville by a behavioral neurologist (NGR) came to autopsy. Most patients were followed longitudinally. Of these 221 cases, 13 did not have a diagnosis of dementia and 3 had Creutzfeldt-Jakob disease, all of which were excluded from this study. Of the remaining 205 cases 43% were women and the median age of death was 79 years, range 37 to 99 years. There were 28 cases with HpScl, and their medical records were reviewed for clinical characteristics, including symptoms at onset and later in the course.

Pathological and genetic analyses

The brains were evaluated in a systematic and standardized manner as previously described.[15] The protocol included freezing one-half of the brain and histologic evaluation of the other (usually left) half. Sections from frontal, temporal, parietal and occipital cortices, hippocampus, amygdala, basal ganglia, thalamus, midbrain, pons, medulla and cerebellum were examined with hematoxylin and eosin (H&E). Sections of cortex, hippocampus, amygdala, basal ganglia and cerebellum were studied with thioflavin-S fluorescent microscopy, and the density of senile plaques and NFT was recorded in these sections. Sections were also processed with immunohistochemistry for ubiquitin,[16] phospho-tau,[17] TDP-43[18] and FUS,[19] as indicated. We screened for the presence of TDP-43 positive pathology in all cases in a section of medial temporal lobe that contained hippocampus, parahippocampal gyrus, occipitotemporal gyrus and inferior temporal gyrus. If present, additional sections were studied, including midfrontal cortex, amygdala, basal ganglia, midbrain and medulla. TDP-43-positive cases were sub-classified as reported previously,[20] using a scheme originally devised by Mackenzie and colleagues.[21] A Braak NFT stage[22] was assigned to all cases based upon the distribution of NFTs with thioflavin-S fluorescent microscopy. We considered cases with frequent neuritic plaques and a Braak NFT stage greater than III as having AD[23] regardless of other concurrent pathologic processes. All cases were screened for Lewy bodies in the amygdala/basal forebrain with α-synuclein immunohistochemistry.[24] FTLD-U was diagnosed if there was evidence of neuronal loss, gliosis and spongiosis in frontal and temporal cortices, ubiquitin-positive intraneuronal inclusions and abnormal neurites.[25] Presence of HpScl was diagnosed if neuronal loss and

gliosis was selective for CA1 and/or the subiculum in the absence of other pathologic findings that could account for neuronal loss in this region. In cases with NFTs, the degree of neuronal loss and gliosis had to be disproportionate to the density of NFTs, especially extracellular NFTs.[1]

GRN rs5848 SNP and *APOE* genotyping was performed on all cases with available DNA from frozen brain using ABI Taqman assays as previously described.[10, 11]

Statistical Analyses

Thirteen cases did not have precise ages of onset in which case our best estimates were used for summaries. Importantly, for all of the HpScl cases there was no uncertainty regarding whether ages of onset were above or below 75 years. Fisher's exact test was used to assess simple associations, and exact binomial confidence intervals were constructed for selected proportions. The Mann-Whitney test was used to compare age of death according to presence of HpScl. Logistic regression was used to estimate odds ratios with 95% confidence intervals and to obtain p-values to summarize the strength of evidence for associations. No adjustments were made for multiple testing in these exploratory analyses.

RESULTS

Frequency of HpScl

In this consecutive autopsy series of 205 dementia patients, 28 had HpScl (14%, 95% CI 9 to 19%), and 25 of the 28 cases (89%; 95% CI: 72 to 98%) had TDP-43 positive inclusions. The three cases that were TDP-43 negative all had onset before age 75. One had FUS (fused in sarcoma) positive inclusions, one had significant cerebrovascular disease as well as Lewy body disease and AD, and the third had severe AD with pallidonigral degeneration. HpScl was present in 65% (14/22) of cases with FTLD-U, 22% (10/45) of AD cases with TDP-43 pathology, and 2% (2/112) of AD cases without TDP-43 pathology. HpScl was present in 37% (25/68) of all cases with TDP-43 pathology. Patients with HpScl had a later age at death (median 83 years versus 79 years, p=0.005). The observed frequency of HpScl among those with age of onset greater than 75 years was 21% (11/53) compared to 11% (17/152) in those with age of onset less than 75 years although not statistically significant (p=0.1).

Clinical features of HpScl

The HpScl cases with TDP-43 pathology were divided into two groups - onset before and after age 75. For those with onset before age 75 (n=14), 6 presented with features of FTD with personality change and executive dysfunction (n=1), language difficulty (n=3) or both (n=2); 7 had predominant amnestic features that were clinically diagnosed with AD; and one had visual hallucinations and parkinsonism consistent with clinically probable DLB. All patients with a clinical diagnosis of FTD had autopsy findings consistent with FTLD-U, and those with clinically probable AD had pathologic findings consistent with AD, often with mixed pathology such as Lewy bodies or vascular disease. For cases presenting after age 75 (n=11), 8 had an amnestic syndrome and were diagnosed with clinically probable AD. Of these 8 cases with clinically probable AD, 2 had pathologic findings of AD, but the remaining 6 did not meet criteria for high probability AD (Braak stage III or less), and all were TDP-43 positive. The amnestic patients not meeting AD pathologic criteria included 4 with FTLD-U, 1 with diffuse Lewy body disease and 1 with no other significant pathology ("pure HpScl") (see Tables 1 and 2). However we have used a Fisher's exact test to compare the proportions of patients with pathological AD in those with clinical diagnosis of AD and having HpScl in those with age of onset above and below 75 and found a significant difference (see Table 3).

TDP-43 pathology and genetic findings

HpScl has been reported to occur frequently in autopsy patients having FTLD-U and in AD with TDP-43.[2, 4–6] We compared the frequency of TDP-43 positivity in cases with HpScl to those without. We found 25 of 28 (89%) with HpScl were TDP-43 positive, compared to 43 of 177 (24%) HpScl negative patients, a highly significant difference ($p<0.001$).

Patients with *GRN* mutations have low levels of progranulin and at autopsy have TDP-43 pathology.[8] It has been found that the T-allele of rs5848 in the *GRN* 3′UTR is associated with decreased progranulin expression.[11] In order to study if rs5848 is associated with HpScl, we analyzed rs5848 in patients with HpScl (n=25 after excluding 2 cases with *GRN* mutations and 1 with no sample) and controls without HpScl (n=176, excluding 1 with no sample). Assuming additive dosage for the T-allele in a logistic regression model, we identified significant association ($p<0.001$) with an estimated odds ratio associated with each copy of the T-allele of 3.0 (95% CI 1.5 to 5.8) (Table 4). Although the frequency of the APOE ε4 allele was lower in cases with HpScl (11/26, 42%, 2 with no samples) than those without (102/176, 58%, one with no sample), this was not statistically significant ($p= 0.14$).

DISCUSSION

In this consecutive series of elderly patients with dementia who came to autopsy from 1997–2008, HpScl was detected in 14% of the cases, which makes this a common pathologic finding most often associated with other pathologic processes. However, this study population consisted of patients referred to a tertiary center, therefore cannot fully represent the general random elderly population with dementia due to selection bias. This may explain why the frequency in our study is slightly higher than reported in community based sample which is between 3% to 13%[2]. Several published studies have shown that patients with HpScl in the elderly tend to have a later onset than those without HpScl[2, 26, 27]. This is also the case in the present series. In patients with symptom onset 75 years of age or later, 21% (11/53) had HpScl at autopsy compared to 11% (17/152) of those with onset before 75 years of age. There was a high concordance between clinical diagnosis and pathologic diagnosis of HpScl patients with onset before 75 (e.g., behavioral or language features associated with FTLD-U pathology, and amnestic features with AD pathology). In those whose dementia began after age 75, the most common clinical presentation was that of probable AD (8/11), but pathologically most cases (6/8) did not have sufficient NFTs to warrant a diagnosis of AD (Braak stage III or less) and actually had FTLD-U in most cases (4/6). This suggests that FTLD-U presenting with an amnestic syndrome should be in the differential diagnosis of patients having late onset (≥75 years) clinical probable AD. None of these FTLD-U cases had significant vascular disease at autopsy or clinical history of cardiac arrest, which makes hypoxic/ischemic cause of HpScl unlikely. These findings have important implications on patient management and in genetic studies in late onset dementia that use cases with clinically probable AD without ancillary methods (e.g., cerebrospinal fluid analyses for Aβ and tau[28]) to exclude FTLD-U.

Not surprisingly, 89% (25/28) of the HpScl cases were positive for TDP-43 inclusions. Other studies have shown that HpScl occurs in 75% of TDP-43 positive FTLD-U cases[6], and in those with both AD and TDP-43 pathology 31% have HpScl, whereas in those with AD only HpScl occurs in 4%[2]. In our series, HpScl is present in 64% (14/22) of cases with FTLD-U, 22% (10/45) of AD cases with TDP-43 pathology, and 2% (2/112) of AD cases without TDP-43 pathology. HpScl was also present in 37% (25/68) of all dementia cases with TDP-43 inclusions. We find that TDP-43 pathology is highly associated with HpScl in our group which suggests that TDP-43 has a strong association with HpScl in neurodegenerative diseases.

GRN mutations may cause TDP-43 pathology[7–9]. The mutations produce null alleles, and cause at least 50% decrease in progranulin expression. Progranulin expression in the brain is also associated with a common variant in the 3′UTR of *GRN*, in a putative microRNA binding site[11]. In a recent study of HpScl in a series of 644 autopsy-confirmed AD, 57 had HpScl, and 72% of the HpScl cases carried a T-allele, while it was present in 51% of AD without HpScl (p =0.005). The results suggested that the T-allele may be a risk factor for HpScl in the elderly[14]. In our smaller autopsy series that included a mixture of neurodegenerative processes, including some cases of FTLD-U, there was also a statistically significant association of the T-allele with HpScl (p=0.008).

Conclusion

This consecutive autopsy series shows a 14% (28/205) prevalence of HpScl and its strong association with TDP-43 pathology. Ours is one of the first studies to emphasize that patients with late onset (75 years of age or older) dementia who present with a predominantly amnestic syndrome similar to those of clinically probable AD can have FTLD-U with HpScl, not AD. Diagnostic accuracy was considerably better in those with early onset dementia. Presumably, the severe neuronal loss and gliosis in the medial temporal lobe structures is the correlate of the amnestic syndrome, and it suggests that medial temporal lobe structures may have been the initial focus of the pathologic process. It remains to be determined if ancillary diagnostic methods, such as amyloid imaging or CSF biomarkers, can differentiate FTLD-U with HpScl from AD in late onset dementia patients with predominant amnestic syndrome. Making this distinction may have a significant impact on genetic and treatment studies.

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Table 1

Clinical presentation of HpScl cases with TDP-43 pathology [a]

Age of onset:		**< 75 years**		**≥ 75 years**	
Braak Stage:		**≤ III**	**> III**	**≤ III**	**> III**
Clinical Dx:	**AD** [b]	1	6	6	2
	FTD [c]	5	1	2	0
	Other	0	1 [d]	1 [d]	0

[a] The 3 non TDP-43 cases, one had significant vascular disease, one with atypical FTLD with FUS positive inclusions and one had AD, with age of onset < 75

[b] Patients presented with a predominant amnestic syndrome

[c] Patients with onset before 75 (including one clinical CBD with Braak stage ≤ III) presented with personality change or executive dysfunction (n=1), aphasia (n=3) or both (n=2)

[d] Clinical diagnosis of DLB

Table 2

Pathologic diagnoses in HpScl cases with TDP-43 pathology

Age of onset:		**< 75 years**		**≥ 75 years**	
Braak Stage:		**≤ III**	**> III**	**≤ III**	**> III**
Pathologic Dx:	**AD**	0	7 [a]	0	2
	FTLD-U	5	1 [c]	7 [d]	0
	Other	1 [b]	0	2 [e]	0

[a] Four with significant vascular disease, one with diffuse Lewy body disease

[b] Diffuse Lewy body disease with atypical AD and cerebral amyloid angiopathy

[c] With significant vascular disease

[d] The case with clinical diagnosis of DLB had FTLD-U at autopsy

[e] One diffuse Lewy body disease with mixed vascular pathology, one “pure HpScl”

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Table 3

Pathology findings for subjects with a clinical diagnosis of AD and with HpScl according to age of onset.

		Pathologic Diagnosis		
		AD [a]	**FTLD-U**	**Other**
Age of onset	**< 75 years**	8 [b]	1	0
	≥ 75 years	2	4	2

[a] Fisher's exact test yielded p=0.015 in comparison of proportion of patients with pathologic AD in older versus younger age group.

[b] Two of the subjects in this cell did not have TDP-43 pathology; all others in table had TDP-43 pathology.

Table 4

Association of *GRN* rs5848 with HpScl [a]

	***GRN* rs5848 genotype**		
	CC	**CT**	**TT**
HpScl	6 24%	12 48%	7 28%
No HpScl	88 50%	77 44%	11 6%

[a] Does not include 2 patients with GRN mutations or 2 patients with no sample.

[b] Estimated odds ratio for HpScl associated with T allele (additive dosage model) is 3.00 (95% CI: 1.54 to 5.84, p<0.001).