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Progressive amnestic dementia, hippocampal sclerosis, and mutation in *C9ORF72*

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Abstract The most common cause of familial frontotemporal lobar degeneration with TAR DNA-binding protein-43 pathology (FTLD-TDP) has been found to be an expansion of a hexanucleotide repeat (GGGGCC) in a noncoding region of the gene *C9ORF72*. Hippocampal sclerosis (HpScl) is a common finding in FTLD-TDP. Our objective was to screen for the presence of *C9ORF72* hexanucleotide repeat expansions in a pathologically confirmed cohort of "pure" hippocampal sclerosis cases ($n = 33$), outside the setting of FTLD-TDP and Alzheimer's disease (AD). Using a recently described repeat-associated non-ATG (RAN) translation (C9RANT) antibody that was found to be highly specific for c9FTD/ALS, we identified a single "pure" HpScl autopsy case with a repeat expansion in *C9ORF72* (c9HpScl). Mutation screening was also performed with repeat-primed polymerase chain reaction and further confirmed with Southern blotting. The c9HpScl patient had a 14-year history of a slowly progressive amnestic syndrome and a clinical diagnosis of probable AD. Neuropsychological testing revealed memory impairment, but no deficits in other cognitive domains. Autopsy showed hippocampal sclerosis with TDP-43 immunoreactive neuronal inclusions relatively limited to limbic lobe structures. Neuritic pathology immunoreactive for p62 was more frequent than TDP-43 in amygdala and hippocampus. Frequent p62-positive neuronal inclusions were present in cerebellar granule neurons as is typical of *C9ORF72* mutation carriers. There was no significant FTLD or motor neuron disease. C9RANT was found to be sensitive and specific in this autopsy-confirmed series of HpScl cases. The findings in this patient suggest that the clinical and pathologic spectrum of *C9ORF72* repeat expansion is wider than frontotemporal dementia and motor neuron disease, including cases of progressive amnestic dementia with restricted TDP-43 pathology associated with HpScl.

Keywords Hippocampus · *C9ORF72* · Memory · Neuropathology · Frontotemporal lobar degeneration · C9RANT

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Introduction

Hippocampal sclerosis (HpScl) in the elderly is characterized by selective neuronal loss and gliosis in CA1 sector and subiculum of the hippocampus, often associated with a slowly progressive amnestic syndrome [40]. HpScl is not uncommon in autopsy series of dementia of the elderly [13, 35]. The pathogenesis of HpScl is unknown, but it is hypothesized to be neurodegenerative and often associated with neuronal inclusions containing TAR DNA-binding protein-43 (TDP-43) [3]. Neuronal loss in the same distribution can also be seen in patients with hypoxic–ischemic injury, but this is not associated with TDP-43 pathology [3, 20]. HpScl is frequent in other

degenerative disorders, especially frontotemporal lobar degeneration with TDP-43 pathology (FTLD-TDP) [18]. When HpScl is the only neuropathologic finding sufficient to cause dementia, such that it cannot be explained by FTLD or Alzheimer's disease (AD) pathology, it is considered "pure" HpScl [2].

Investigation of genetic correlates of HpScl is a relatively new area of research. Mutations in the gene for progranulin (*GRN*) cause FTLD-TDP [5], while a genetic variant in the 3′-UTR of *GRN* in a putative micro-RNA binding site (SNP rs5848, T minor allele) is a risk factor not only for FTLD-TDP [37], but also for HpScl [12]. Pathologically, many individuals harboring *GRN* mutations have concomitant HpScl pathology [23]. Identification of disease-causing mutations in *GRN* still could not account for a genetic substrate underlying a significant proportion of autosomal dominant FTLD-TDP cases. Results from linkage analyses in these families revealed a locus on the short arm of chromosome 9 [8, 15, 19, 22, 27, 30]. The most common genetic cause of FTLD-TDP was finally identified in 2011 and found to be an expansion of a hexanucleotide repeat (GGGGCC) in a noncoding region of the gene *C9ORF72* [11, 38]. The current recommended terminology for frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) caused by mutations in *C9ORF72* is c9FTD/ALS [11]. The clinical presentation of most patients with c9FTD/ALS is that of behavioral variant FTD, FTD with motor neuron disease or ALS, but other clinical phenotypes are also reported (e.g., psychosis or progressive aphasia) [16].

In an autopsy series of c9FTD/ALS, concomitant HpScl was frequent [31]. In addition, these cases presented with the characteristic c9FTD/ALS neuropathologic phenotype, namely, ubiquitin-positive, TDP-43-negative neuronal cytoplasmic inclusions in the cerebellum, hippocampus, and neocortex which are immunoreactive for ubiquitin-binding proteins sequestosome-1/p62 and ubiquilin-2 [7]. Recently, our group and a second independent group uncovered an unconventional form of translation of the GGGGCC expanded repeat known as repeat-associated non-ATG (RAN) translation which leads to the generation of three alternating copolymer peptides [4, 29]. This pathology, termed C9RANT, is highly specific to the central nervous system of c9FTD/ALS [4].

In this study, our goals were to (1) screen pure HpScl cases with C9RANT immunohistochemistry [4] and (2) validate the results with genetic methods [11], to identify the frequency of pure HpScl cases harboring the *C9ORF72* repeat expansion. The findings presented in this report contribute to increasing evidence that the clinical and pathologic spectrum of disorders in *C9ORF72* is wider than previously suggested.

Materials and methods

Case material

The brain bank for neurodegenerative disorders at Mayo Clinic Jacksonville was queried for all cases with a pathologic diagnosis of HpScl with available frozen tissue for DNA extraction (n = 280). Cases were excluded if there was a coexisting neuropathologic diagnosis of FTLD (n = 73), AD (n = 138), or Lewy body disease (n = 16). We also excluded other significant neurodegenerative diseases, such as progressive supranuclear palsy, multiple system atrophy, and corticobasal degeneration (n = 22). A total of 33 cases met inclusion criteria and only 1 case was found to have a *C9ORF72* repeat expansion (c9HpScl). The clinical information of the c9HpScl case was abstracted from the medical records.

Neuropathologic methods

At the time of neuropathologic examinations, brains were submitted for evaluation with the left hemibrain fixed in formalin and the right frozen at −80°. Fixed brain weight was calculated based upon doubling the weight of the left hemibrain. Using standardized dissection and sampling methods described previously [31], tissue samples were processed and embedded in paraffin blocks. The posterior hippocampus was used to evaluate C9RANT and TDP-43 immunoreactivity in all cases. Immunohistochemistry was performed on a DAKO Autostainer (Universal Staining System Carpinteria, California) with the pooled-peptide C9RANT antibody (Rb5823 1:5000, Mayo Clinic antibody) [4] and phospho-Serine 409/410 TDP-43 (1:5,000 mouse monoclonal; Cosmo Bio Co., LTD.). Antigen retrieval was performed by steaming in deionized water for 30 min. To further characterize the *C9ORF72* mutation case, sections of frontal, temporal, parietal and motor cortex, hippocampus, amygdala, basal ganglia, thalamus, midbrain, medulla, and cerebellum were evaluated using additional histologic methods. Additional immunohistochemical evaluations included: IBA1 (1:3,000 rabbit anti-ionized calcium binding adaptor molecule 1, Wako Chemicals), Ubi1 (1:40,000 mouse monoclonal anti-ubiquitin, Millipore), p62 (1:250 mouse monoclonal anti-p62/sequestosome; BD Transduction Laboratories), Ubqln2 (M03-5F5, 1:60,000, Abnova, Taipei City, Taiwan), CP13 (1:1,000 mouse monoclonal anti phospho-tau, gift from Peter Davies), collagen IV (1:1,000 rabbit polyclonal anti-Collagen IV, Rockland Inc.), and alpha-synuclein (1:3,000 rabbit polyclonal anti-alpha-synuclein, Mayo Clinic antibody). Steam was used for antigen retrieval for each of the additional antibodies, except for alpha-synuclein, which was incubated on slides for 30 min in 98 % formic acid, followed by steaming in

deionized water for 30 min. Senile plaques and neurofibrillary tangles (NFT) were counted and the severity of amyloid angiopathy scored with thioflavin S fluorescent microscopy, as previously described [32]. At the time of diagnosis, a Braak NFT stage was assigned using thioflavin S [9]. The neuritic plaque score was assigned by retrospectively assessing neuropathologic reports to determine distribution on thioflavin S stains of the most affected cortices according to the recent neuropathologic guidelines recommended by the National Institutes on Aging and the Alzheimer's Association [17, 28]. We used a semi-quantitative score that assessed the distribution as none, sparse, moderate, and frequent.

The C9RANT antibody detects discrete neuronal inclusions, which were used to screen for C9RANT positivity throughout hippocampal subsectors and medial temporal cortex. The TDP-43 antibody was used to screen for positivity, as well as classify previously described harmonized TDP-43 subtypes A–C [24].

Genetic methods

Genomic DNA was extracted from frozen brain tissue and screened for the *C9ORF72* expanded hexanucleotide repeat using repeat-primed PCR and Southern blotting techniques as previously described [11]. Briefly, an initial assay of GGGGCC hexanucleotide repeat expansion was amplified for each case ($n = 33$) using one fluorescently labeled PCR primer. Next, using an automated ABI3730 DNA analyzer, a fragment length analysis was performed using GeneMapper software (Applied Biosystems). If a case appears homozygous in the initial assay, it was then analyzed using a repeat-primed PCR method. An individual with a repeat expansion will show a characteristic stutter amplification pattern on electropherogram. For Southern blotting, DNA was extracted from frozen brain tissue. Genomic DNA (10–15 μg) was digested with XbaI and separated by electrophoresis for 6 h at 100 V in a 0.8 % agarose gel. Hereafter, DNA was transferred to a positively charged nylon membrane (Roche, Penzberg, Upper Bavaria, Germany) and cross-linked by UV irradiation. Following prehybridization in digoxigenin (DIG) EasyHyb solution (Roche), hybridization was performed with a DIG-labeled probe [11]. To detect the probe an anti-DIG antibody (1:10,000, Roche) was used, which was visualized with CDP-star substrate (Roche) on X-ray film after exposures of 30–60 min.

Results

Pathogenetic screen

Of the 280 cases identified, 33 (12 %) were not found to have coexisting FTLD, AD, or other significant neurodegenerative diseases. The demographics and pathologic information are summarized in Table 1. Of the final 33 HpScl, there were 22 TDP-43 positive cases, 10 TDP-43 negative cases, and one case that lacked fixed tissue. Regardless of TDP-43 positivity, each case was screened with C9RANT in the hippocampus. One case was found to have C9RANT positivity with the highest burden of inclusions found in the dentate fascia and endplate (CA4). Occasional neuronal cytoplasmic inclusions (NCI) and "pre-inclusions" with granular somatodendritic C9RANT immunoreactivity could be found in the medial temporal lobe cortex (Fig. 1a, b).

Blinded to the C9RANT results, we found 1 out of the 33 cases with the characteristic stutter pattern in the repeat-primed PCR assay, indicative of an expansion in *C9ORF72* (Fig. 2a). Southern blot using DNA extracted from cerebellum was performed to confirm the presence of a pathogenic repeat expansion, which highlighted a smear of high-molecular weight bands (Fig. 2b). The C9RANT antibody identified the same case that was found to have a *C9ORF72* repeat expansion using genetic methods. In this series of HpScl cases that lacked neuropathology consistent with FTLD or advanced AD diagnoses, the C9RANT antibody was 100 % sensitive and specific.

Table 1 Summary of demographics and neuropathology in hippocampal sclerosis

All data are presented as median (25th, 75th interquartile range) unless noted otherwise. One case lacked fixed tissue and therefore could not be evaluated for TDP-43 and is not presented in this table. Acronyms: *HpScl* hippocampal sclerosis, *TDP-43* TAR DNA-binding protein-43, *g* grams

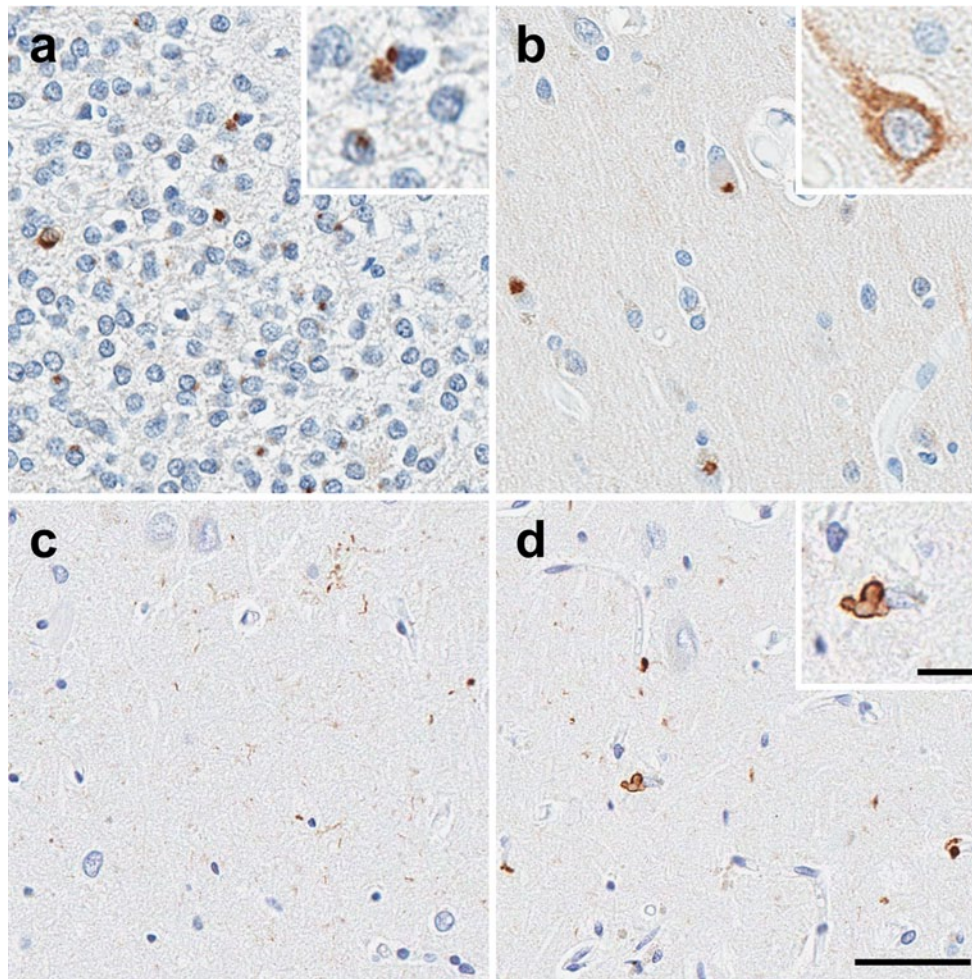|	c9HpScl	HpScl TDP-43 positive	HpScl TDP-43 negative
Sample size, *n*	1	21	10
Sex, males (%)	1 (100)	9 (43)	3 (30)
Age at death, years	76	91 (87, 94)	88 (84, 93)
Brain weight, g	1140	1160 (1095, 1220)	1091 (978, 1250)
Braak NFT stage	III	II (II, III)	III (I, IV)
Neuritic plaque score	0	0	0 (0, 0.5)
TDP-43 positivity	Positive	Positive	Negative
Type A (1), *n* (%)	–	17 (81)	–
Type B (3), *n* (%)	1 (100)	4 (19)	–
Type C (2), *n* (%)	–	–	–

Fig. 1 C9RANT immunohistochemistry in the hippocampus (**a**) shows the highest number of neuronal cytoplasmic inclusions (NCI, inset) in the dentate fascia. (**b**) Occasional NCIs and "pre-inclusions" with granular somatodendritic C9RANT immunoreactivity (inset) could be found in the medial temporal lobe cortex. TDP-43 immu- nohistochemistry in the CA1 sector of the hippocampus shows (**c**) synaptic or fine neurites in areas of relative neuronal preservation, as well as (**d**) TDP-43-positive perivascular glial inclusions. They can be seen as a bi-lobed inclusion (inset) or as dense perivascular deposits (*arrowhead*) (*bar* 50 μm **a–d**, *insets* 100 μm)

Clinicopathological summary

Here, we give a clinicopathologic summary of the c9Hp- Scl case identified in the pathogenetic screen. The c9HpScl case was a 76-year-old man with a final diagnosis prior to death of clinically probable AD, initially evaluated in 1995 at 72 years of age with a 10-year history of slowly progres- sive episodic memory impairment (age of onset 62 years). The wife reported no change in personality or behavior, "He was socially active and plays piano, bridge, reads, walks, and shoots pool". His mother had slowly progressive amnestic dementia with onset in her 70s and death at age 96. There was no other family history of dementia or ALS. His past medical history was notable for coronary artery disease with a four-vessel coronary artery bypass graft in 1987. Cranial nerves were unremarkable, his palate moved symmetrically, and his tongue was midline. Although there were no EMG studies conducted, motor testing revealed preserved strength in the upper and lower extremities. Word fluency was within normal limits, naming 13 words with the letter "F" [6]. A Mini-Mental State Examination [14] revealed difficulties with the date, month, and day of the week. He also had mild difficulties with recall and scored a total of 24 out of 30. There were no signs of para- noia or psychosis. Neuropsychological evaluation showed executive/frontal functioning within normal limits and severe impairment in memory, especially for delayed recall (Table 2). Specific neuropsychological tests designed for the diagnosis of FTD were not conducted. Magnetic reso- nance imaging in 1995 showed diffuse cerebral volume loss and white matter changes consistent with the age-asso- ciated radiologic finding, leukoaraiosis [33]. There was no
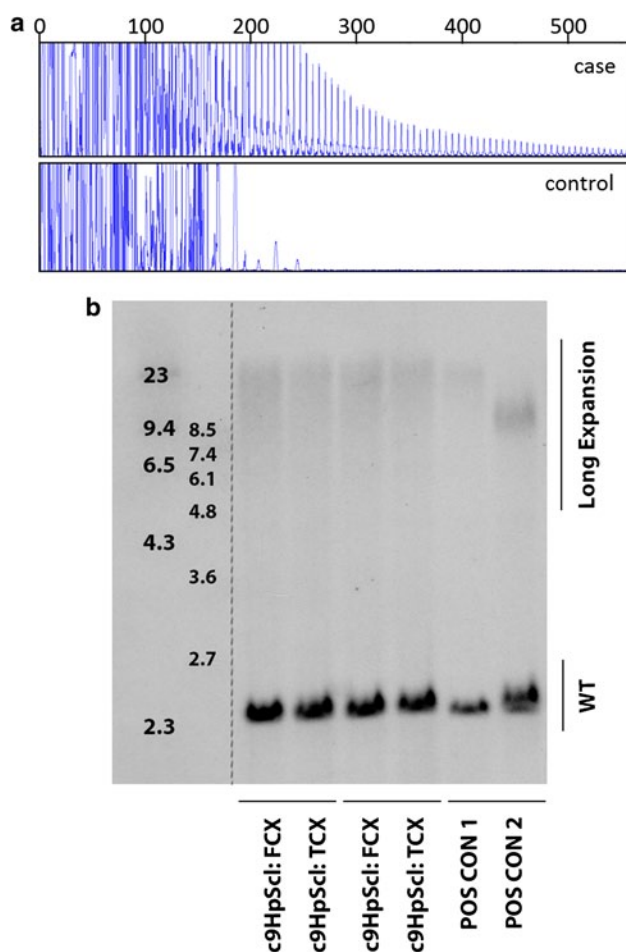
Table 2 Neuropsychological evaluation of *C9ORF72*-associated HpScl case (september 1995)

Test/subtest	Percentile	Score
Wechsler Adult Intelligence Scale—revised*		
Digit span	98	16
Vocabulary	95	15
Comprehension	84	13
Similarities	75	12
Block design	37	9
Digit symbol	25	8
Wechsler Memory Scale—revised		
Verbal memory	2	70
Visual memory	7	77
General memory	1	68
Attention/concentration	91	120
Delayed recall	1	67
Trail making test B	75	N/A
Beck depression inventory	N/A	4

HpScl hippocampal sclerosis, *n/a* test not available in clinical records

Fig. 2 a The electropherogram plot of repeat-primed PCR reaction products zoomed to 2,000 relative fluorescence units to show stutter amplification. Our case and a control (*C9ORF72*-negative) with HpScl are graphically represented in the upper and lower panels, respectively. **b** Southern blot of two repeat expansion carriers. *Lane 1* shows DIG-labeled DNA Molecular Weight Marker II (Roche) with fragments between 2,322 and 23,130 bp. *Lane 2* shows DIG-labeled DNA Molecular Weight Marker VII (Roche) with fragments between 2,799 and 8,576 bp. Duplicate measurements of frontal cortex (*lanes 3* and *5*) and temporal cortex (*lanes 4* and *6*) from the chromosome 9 HpScl case show an expanded allele with repeat sizes up to ~23 kb (>3,000 repeat units), in addition to the wild-type allele of ~2.3 kb. Note the smear of high-molecular weight bands, suggestive of somatic instability. Frontal cortex (*lane 7*) and cerebellum (*lane 8*) of an ALS patient with a *C9ORF72* repeat expansion are shown as a positive control

evidence of infarction or abnormal enhancement with the medial temporal lobe. The patient was further lost to follow-up in the 5 years preceding his death.

The calculated fixed brain weight was 1,140 g (Table 1). There was mild cortical atrophy and ventricular dilation, especially in the temporal horn of the lateral ventricle. The hippocampal formation, but not the amygdala, was atrophic (Fig. 3a). The cerebellum was unremarkable. The substantia nigra and locus coeruleus had visible neuromelanin pigment. Microscopic inspection of the substantia nigra and locus coeruleus showed a normal neuronal population with no NFTs or Lewy bodies. There was severe neuronal loss and gliosis that was selective for CA1, with less neuronal loss in the subiculum and preservation of CA2/3, endplate and dentate fascia (Fig. 3d). Senile plaques were not detected in any cortical region, hippocampus, amygdala, or cerebellum. There were only a few isolated NFTs in the frontal and temporal cortices and in the hippocampus and amygdala, but more numerous NFTs in the entorhinal cortex. As expected, no NFT pathology was found in the cerebellum. There was minimal evidence of atherosclerosis, but no arteriosclerosis was noted. White matter rarefaction was consistent with age [34]. There was no evidence of lacunar infarcts, microscopic infarcts, or ischemic gliosis. Mild amyloid angiopathy was detected in the frontal and occipital cortices.

Phospho-TDP-43 immunohistochemistry showed NCIs in the dentate gyrus of the hippocampus (Fig. 3b), but only rare NCIs in the frontal and temporal cortices and no significant cortical neuronal loss or gliosis (Table 3). There were sparse TDP-43-positive dystrophic neurites and no neuronal intranuclear inclusions (NII) in the hippocampus, consistent with Type B [24]. The presence of hippocampal synaptic or fine neurites in the CA1 (Fig. 1c) was also noted in areas of relative neuronal preservation, a previously reported feature of many *C9ORF72*-associated FTLD cases [31]. TDP-43-positive perivascular glial inclusions (Fig. 1d), considered to be abnormal inclusions in astrocytic end-feet processes [21], were present in the hippocampus and entorhinal cortex, but sparse in the frontal

Fig. 3 **a** Macroscopic findings in c9HpScl show atrophic hippocampus (*arrow*) in the CA1 region. **b** TDP-43 staining of the hippocampus shows few neuronal cytoplasmic inclusions (NCIs) and no intranuclear inclusions (NIIs). **c** p62 immunohistochemistry of the same region shows many NCIs and scattered NIIs in dentate fascia. **d** An H&E of the anterior hippocampus shows focal CA1 and subiculum pathology, while CA2/3 pyramidal neurons remain resistant (*arrow* shows transition to neuronal dropout). Macroscopic findings correlate with underlying neuronal loss and gliosis (*inset* shows higher magnification of neuronal loss and gliosis characteristic of HpScl in of CA1) (*bar* 45 µm for B&C at 40× magnification, 1,150 µm for D at 1× magnification, and 90 µm for *inset* at 20× magnification)
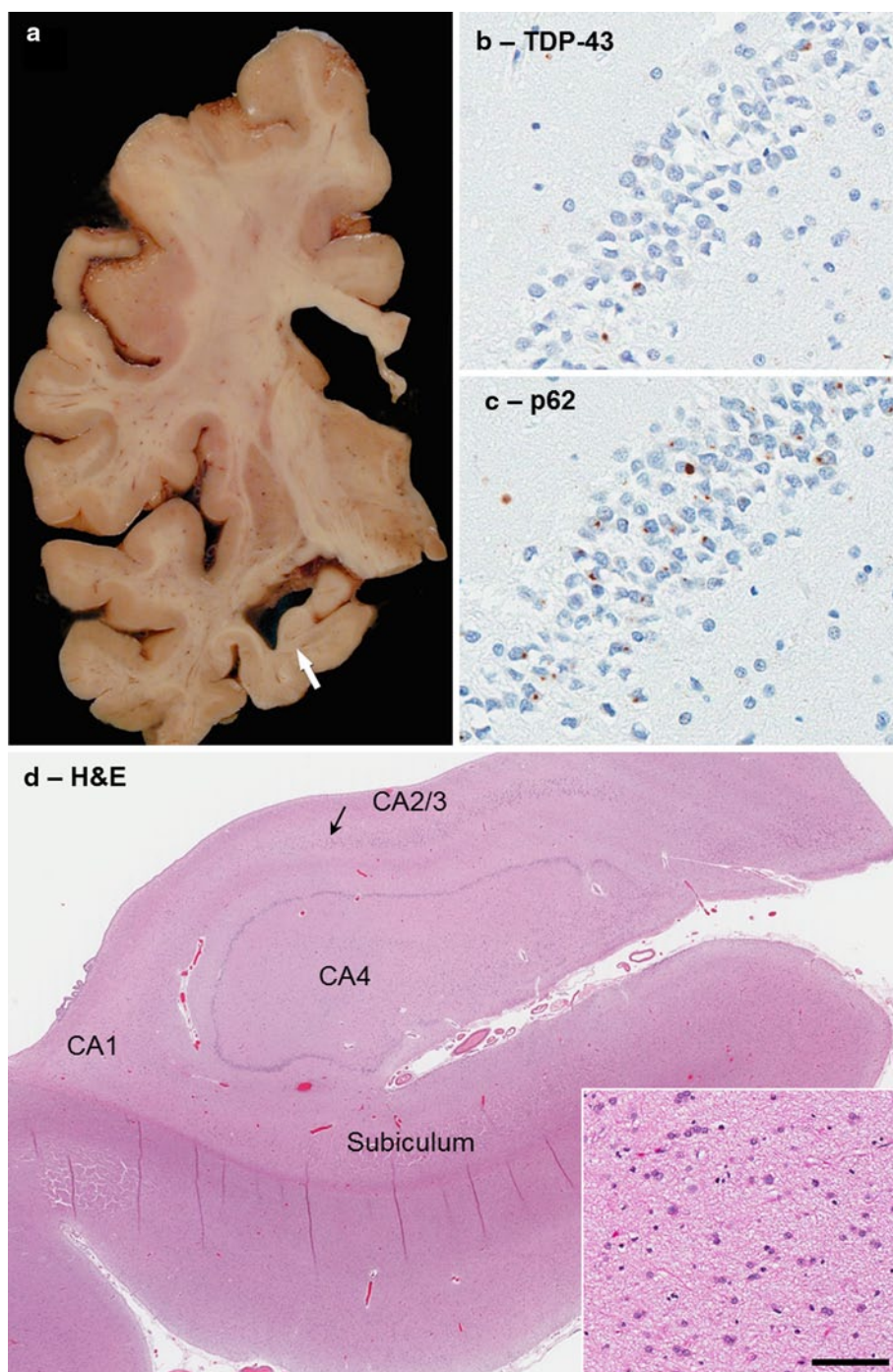
and temporal cortices and amygdala. CA1 and nucleus accumbens showed the highest density of the perivascular TDP-43 inclusions (Table 3). No evidence of motor neuron degeneration was present. We did not find TDP-43 pathology, neuronal loss, or Bunina bodies in the spinal cord.

In addition to TDP-43 positive pathology, there were also TDP-43-negative, p62/sequestosome-1-positive NCIs and dystrophic neurites (Fig. 3c). In all cortical and subcortical regions evaluated, p62-positive pathologic inclusions were more numerous than TDP-43-positive inclusions, as reported recently by others in c9FTD/ALS [1]. TDP-43 pathology was absent in the cerebellum, but C9RANT immunopositivity showed many NCIs in granule cells and occasionally in Purkinje cells (Fig. 4a, b). As in other previously reported *C9ORF72* repeat expansion cases [1, 10], markers of the ubiquitin–proteasome system (ubiquitin, p62, ubiquilin-2) revealed NCIs in both granule cells and Purkinje cells of the cerebellum (Fig. 4c, d). The density of

Table 3 TDP-43 and C9RANT semi-quantitative assessment across brain regions in c9HpScl

	TDP-43	C9RANT
Hippocampus		
Dentate gyrus	++ NCIs	++/+++ NCIs
CA1	++ Perivascular > fine neurites	−/+
Nucleus accumbens	+++ Perivascular	−
Globus pallidus	−	−
Putamen	−/+	−/+ PreNCIs
Thalamus		
Anterior thalamus	+ Perivascular >NCI	+ NCIs
Medial thalamus	−	+ NCIs
Lateral thalamus	−	+ NCIs
Temporal cortex	+ Perivascular	+ PreNCIs
Frontal cortex	+ Perivascular	+ PreNCIs
Motor cortex	−	−/+ PreNCIs
Parietal cortex	−/+ Perivascular	−
Midbrain		
Substantia nigra	+ Perivascular >skein-like NCIs	−
Red nucleus	+ Perivascular >skein-like NCIs	−
Medulla		
Olive	+ Perivascular >NCIs	−
Cerebellum		
Granule cell layer	−	++ NCIs
Purkinje cell layer	−	+ Star-shaped NCIs

Semi-quantitative scale: −, none; −/+, rare; +, sparse; ++, moderate; +++ severe. *HpScl* hippocampal sclerosis, *TDP-43* TAR DNA-binding protein-43, *NCIs* neuronal cytoplasmic inclusions, *perivascular* globular or bi-lobed perivascular TDP-43 inclusions, *preNCIs* preinclusions with granular somatodendritic C9RANT immunoreactivity in the neuronal cytoplasm

C9RANT pathology was highest in the dentate fascia of the hippocampus and cerebellum, but could be found as rare preinclusions in CA1 of the hippocampus, putamen, and motor cortex (Table 3). Sparse NCIs were found in the thalamus, temporal, and frontal cortex. C9RANT pathology was not observed in nucleus accumbens, globus pallidus, parietal cortex, midbrain, or medulla.

Discussion

This study identified a hippocampal sclerosis case with an expansion in *C9ORF72* that lacked FTLD, AD, or other significant neuropathology. The case was identified using a recently described antibody generated against an unconventional mechanism of translation-repeat-associated non-ATG translation that was validated using repeat-primed PCR and Southern blotting techniques, which was sensitive and specific in this series of HpScl cases. The clinical phenotype in the c9HpScl patient, namely, slowly progressive amnestic syndrome without behavioral or motor neuron signs, is distinctly different from the most common presentations in c9FTD/ALS [16]. Although the final clinical phenotype of this individual was unknown in the 5 years prior to his death, it is that possible he developed FTD, but unlikely given the slowly progressive nature of the disease

course and sparing of frontal cognitive domains at the last neuropsychological examination (i.e., attention/concentration). HpScl is more apt to be clinically misdiagnosed as AD than the behavioral variant FTD [36, 40] and should be considered in the differential diagnosis of patients with *C9ORF72* repeat expansion.

Mutations in *C9ORF72* have been reported in seemingly sporadic cases of FTLD and ALS [11]. The family history of this patient included amnestic disorder in his mother (presenting with symptoms in her 70s), but there was no history of dementia or motor neuron disease in other family members. Although there was no autopsy confirmation, the patient's mother died in her late 90s, which is more than a decade later than our patient. While it is impossible to make any conclusions from these two individuals, it is of interest that the possibility of anticipation, with earlier age of onset in subsequent generations, has been raised in other c9FTD/ALS series [16]. Pathologically, there was selective neuronal loss and gliosis in the hippocampus consistent with HpScl of the elderly, which fits well with the clinical presentation [35, 40]. TDP-43 inclusions were most abundant in limbic structures and sparse in the cortex. The cortical lesions were not associated with cortical neuronal loss or gliosis. Immunohistochemistry for ubiquitin and p62 in *C9ORF72*-associated HpScl of the elderly shows that the pathology can be more widespread than previously
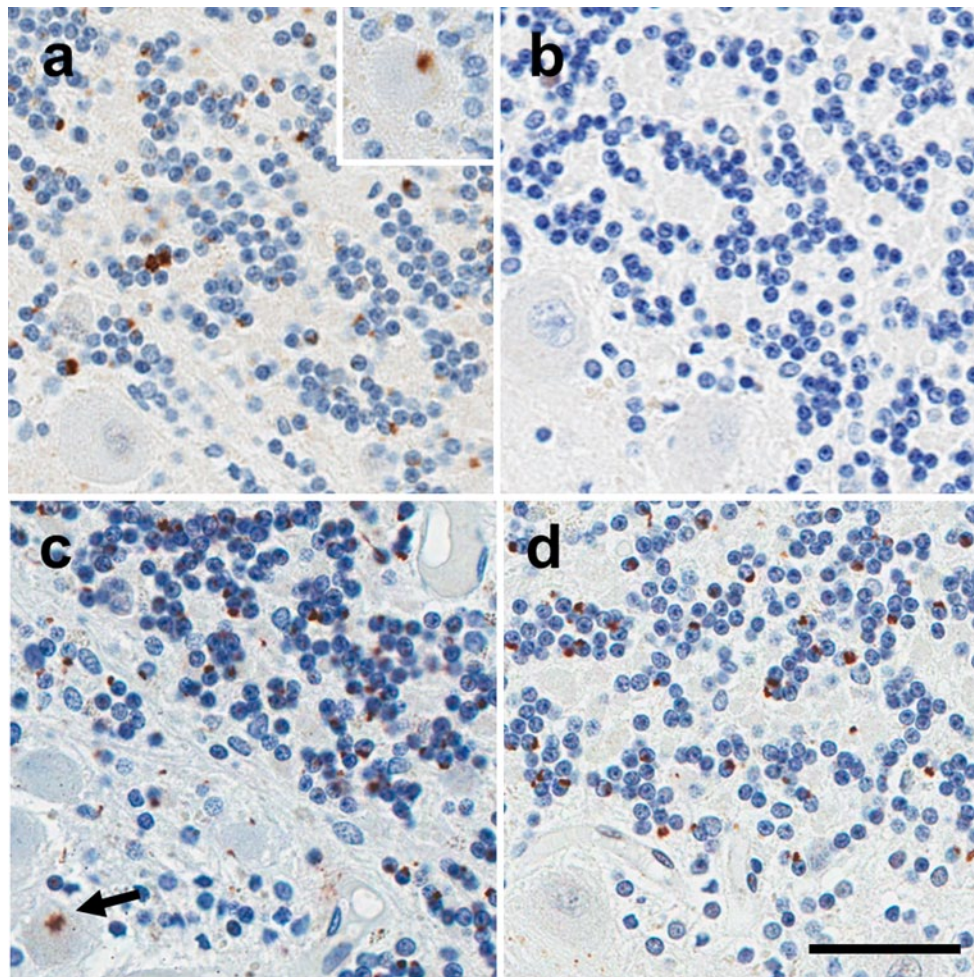
Fig. 4 Immunohistochemistry of the cerebellum in c9HpScl. **a** C9RANT immunopositivity shows many NCIs in granule cells, although occasional Purkinje cells can be observed (*inset*). As expected **b**, the cerebellum lacks TDP-43 pathology. **c** p62 immunohistochemistry shows many NCIs and occasional NIIs of the granule cell layer. A star-shaped NCI (*arrow*) was observed in the Purkinje cell layer of the cerebellum with p62 staining, but not with **d** Ub1 staining. Ub1-positive NCIs were seen at moderate frequency, and no NII were observed (*bar* 45 μm)

suspected based on TDP-43 immunohistochemistry, with NCIs even in the cerebellar cortex. The greater propensity of p62 pathology compared to TDP-43 pathology in *C9ORF72*-associated HpScl suggests that TDP-43 may not be central to the *C9ORF72* disease mechanism, but rather another unknown polyubiquitinated substrate bound by p62. The vast majority of C9RANT lesions are immunopositive for markers of the ubiquitin–proteasome system (ubiquitin, p62, ubiquilin-2, etc.). Preliminary studies have indicated that ~80 % of inclusions would co-label with ubiquitin–proteasome markers and C9RANT serum, ~10 % with ubiquitin–proteasome markers only, and ~10 % with C9RANT only [4]. However, thorough studies have not yet been performed which specifically address the incidence of each C9RANT peptide (GR, GP, and GA) in ubiquitin and ubiquitin-binding protein inclusions. It is worth noting that

the neuropathologic signature of cases with a C9ORF72 hexanucleotide repeat (p62-positive, ubiquilin-2-positive, C9RANT-positive, TDP-43 negative inclusions) offers genetic diagnostic accuracy, whereas other known FTD and ALS mutations such as GRN do not exhibit a distinct neuropathologic phenotype that would permit a similar inference [10].

In our previous clinicopathologic characterization of c9FTD/ALS, we reported that HpScl was common in c9FTLD-TDP (12 of 15), and some patients in this series also presented with amnestic dementia that led to clinical consideration of AD [31]. A large series of familial late-onset clinical AD patients screened for mutations in *C9ORF72* found 6 of 771 patients with mutations [25]. Postmortem analysis of two patients showed pathology consistent with FTLD, with inconsistent concurrent

Alzheimer type neuropathology. There is no mention in this report about whether the two patients with *C9ORF72* mutations and Alzheimer-like clinical presentation had HpScl. The reported frequency of 0.8 % clinical AD patients with *C9ORF72* mutations was consistent with a recent report on a clinical AD patient series from our group, which found 0.9 % of AD patients with an expansion mutation [39]. The frequency of *C9ORF72* in our pathologically confirmed HpScl series is 3.0 % if all cases are considered and 4.5 % if only TDP-43 positive cases are included.

The pathogenesis of HpScl in our patient is unknown, but most evidence favors a neurodegenerative disease process. He had no history of seizures. He had coronary artery disease and a four-vessel coronary artery bypass graft, but there was no history of acute peri-operative cognitive deficits after surgery. The presence of TDP-43 and p62 NCIs and dystrophic neurites in the hippocampus and to a lesser extent in other brain regions supports a neurodegenerative origin for HpScl. It is of some interest that microvascular TDP-43-related pathology was also present [21]. The significance of TDP-43 inclusions in astrocytic end-feet of microvessels remains to be determined; however, we speculate that this pathology may compromise microvascular function and contribute to neuronal loss in regions known to be vulnerable to hypoxic–ischemic injury.

This case adds increasing evidence that the clinical and pathologic spectrum of disorders associated with mutations in *C9ORF72* is wider than merely FTD and ALS. Screening for mutations in *C9ORF72* should be considered in patients with slowly progressive amnestic dementia who have a positive family history, especially if other biomarkers (e.g., apolipoprotein E genotype, neuroimaging, or cerebrospinal fluid analyses) do not support a diagnosis of AD [26]. Further studies are needed to identify the underlying molecular mechanisms responsible for the clinical and pathologic heterogeneity of c9FTLD/ALS.

References

1. Al-Sarraj S, King A, Troakes C et al (2011) p62 positive, TDP-43 negative, neuronal cytoplasmic and intranuclear inclusions in the cerebellum and hippocampus define the pathology of C9orf72-linked FTLD and MND/ALS. Acta Neuropathol 122:691–702
2. Ala TA, Beh GO, Frey WH II (2000) Pure hippocampal sclerosis: a rare cause of dementia mimicking Alzheimer's disease. Neurology 54:843–848
3. Amador-Ortiz C, Ahmed Z, Zehr C, Dickson DW (2007) Hippocampal sclerosis dementia differs from hippocampal sclerosis in frontal lobe degeneration. Acta Neuropathol 113:245–252
4. Ash PE, Bieniek KF, Gendron TF et al (2013) Unconventional translation of C9ORF72 GGGGCC expansion generates insoluble polypeptides specific to c9FTD/ALS. Neuron 77:639–646
5. Baker M, Mackenzie IR, Pickering-Brown SM et al (2006) Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. Nature 442:916–919
6. Benton AL, Hamsher K (1976) Multilingual Aphasia Examination. University of Iowa, Iowa
7. Bieniek KF, Murray ME, Rutherford NJ et al (2013) Tau pathology in frontotemporal lobar degeneration with C9ORF72 hexanucleotide repeat expansion. Acta Neuropathol 125:289–302
8. Boxer AL, Mackenzie IR, Boeve BF et al (2011) Clinical, neuroimaging and neuropathological features of a new chromosome 9p-linked FTD-ALS family. J Neurol Neurosurg Psychiatry 82:196–203
9. Braak H, Braak E (1991) Neuropathological staging of Alzheimer-related changes. Acta Neuropathol 82:239–259
10. Brettschneider J, Van Deerlin VM, Robinson JL et al (2012) Pattern of ubiquilin pathology in ALS and FTLD indicates presence of C9ORF72 hexanucleotide expansion. Acta Neuropathol 123:825–839
11. DeJesus-Hernandez M, Mackenzie IR, Boeve BF et al (2011) Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 72:245–256
12. Dickson DW, Baker M, Rademakers R (2010) Common variant in GRN is a genetic risk factor for hippocampal sclerosis in the elderly. Neurodegener Dis 7:170–174
13. Dickson DW, Davies P, Bevona C et al (1994) Hippocampal sclerosis: a common pathological feature of dementia in very old (>or =80 years of age) humans. Acta Neuropathol 88:212–221
14. Folstein MF, Folstein SE, McHugh PR (1975) "Mini-Mental State". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 12:189–198
15. Gijselinck I, Engelborghs S, Maes G et al (2010) Identification of 2 loci at chromosomes 9 and 14 in a multiplex family with frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Arch Neurol 67:606–616
16. Hodges J (2012) Familial frontotemporal dementia and amyotrophic lateral sclerosis associated with the C9ORF72 hexanucleotide repeat. Brain 135:652–655
17. Hyman BT, Phelps CH, Beach TG et al (2012) National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. Alzheimers Dement 8:1–13
18. Josephs KA, Jones AG, Dickson DW (2004) Hippocampal sclerosis and ubiquitin-positive inclusions in dementia lacking distinctive histopathology. Dement Geriatr Cogn Disord 17:342–345
19. Le Ber I, Camuzat A, Berger E et al (2009) Chromosome 9p-linked families with frontotemporal dementia associated with motor neuron disease. Neurology 72:1669–1676
20. Lee EB, Lee VM, Trojanowski JQ, Neumann M (2008) TDP-43 immunoreactivity in anoxic, ischemic and neoplastic lesions of the central nervous system. Acta Neuropathol 115:305–311

21. Lin WL, Castanedes-Casey M, Dickson DW (2009) Transactivation response DNA-binding protein 43 microvasculopathy in frontotemporal degeneration and familial Lewy body disease. J Neuropathol Exp Neurol 68:1167–1176

22. Luty AA, Kwok JB, Thompson EM et al (2008) Pedigree with frontotemporal lobar degeneration-motor neuron disease and Tar DNA binding protein-43 positive neuropathology: genetic linkage to chromosome 9. BMC Neurol 8:32

23. Mackenzie IR, Baker M, Pickering-Brown S et al (2006) The neuropathology of frontotemporal lobar degeneration caused by mutations in the progranulin gene. Brain 129:3081–3090

24. Mackenzie IR, Neumann M, Baborie A et al (2011) A harmonized classification system for FTLD-TDP pathology. Acta Neuropathol 122:111–113

25. Majounie E, Abramzon Y, Renton AE et al (2012) Repeat expansion in C9ORF72 in Alzheimer's disease. N Engl J Med 366:283–284

26. McKhann GM, Knopman DS, Chertkow H et al (2011) The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement 7:263–269

27. Momeni P, Schymick J, Jain S et al (2006) Analysis of IFT74 as a candidate gene for chromosome 9p-linked ALS-FTD. BMC Neurol 6:44

28. Montine TJ, Phelps CH, Beach TG et al (2012) National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease: a practical approach. Acta Neuropathol 123:1–11

29. Mori K, Weng SM, Arzberger T et al (2013) The C9orf72 GGGGCC repeat is translated into aggregating dipeptide-repeat proteins in FTLD/ALS. Science 339:1335–1338

30. Morita M, Al-Chalabi A, Andersen PM et al (2006) A locus on chromosome 9p confers susceptibility to ALS and frontotemporal dementia. Neurology 66:839–844

31. Murray ME, DeJesus-Hernandez M, Rutherford NJ et al (2011) Clinical and neuropathologic heterogeneity of c9FTD/ALS associated with hexanucleotide repeat expansion in C9ORF72. Acta Neuropathol 122:673–690

32. Murray ME, Graff-Radford NR, Ross OA et al (2011) Neuropathologically defined subtypes of Alzheimer's disease with distinct clinical characteristics: a retrospective study. Lancet Neurol 10:785–796

33. Murray ME, Senjem ML, Petersen RC et al (2010) Functional impact of white matter hyperintensities in cognitively normal elderly subjects. Arch Neurol 67:1379–1385

34. Murray ME, Vemuri P, Preboske GM et al (2012) A quantitative postmortem MRI design sensitive to white matter hyperintensity differences and their relationship with underlying pathology. J Neuropathol Exp Neurol 71:1113–1122

35. Nelson PT, Schmitt FA, Lin Y et al (2011) Hippocampal sclerosis in advanced age: clinical and pathological features. Brain 134:1506–1518

36. Pao WC, Dickson DW, Crook JE et al (2011) Hippocampal sclerosis in the elderly: genetic and pathologic findings, some mimicking Alzheimer disease clinically. Alzheimer Dis Assoc Disord 25:364–368

37. Rademakers R, Eriksen JL, Baker M et al (2008) Common variation in the miR-659 binding-site of GRN is a major risk factor for TDP43-positive frontotemporal dementia. Hum Mol Genet 17:3631–3642

38. Renton AE, Majounie E, Waite A et al (2011) A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron 72:257–268

39. Wojtas A, Heggeli KA, Finch N et al (2012) C9ORF72 repeat expansions and other FTD gene mutations in a clinical AD patient series from Mayo Clinic. Am J Neurodegener Dis 1:107–118

40. Zarow C, Sitzer TE, Chui HC (2008) Understanding hippocampal sclerosis in the elderly: epidemiology, characterization, and diagnostic issues. Curr Neurol Neurosci Rep 8:363–370