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TDP-43 in the hypoglossal nucleus identifies amyotrophic lateral sclerosis in behavioral variant frontotemporal dementia

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ABSTRACT

The hypoglossal nucleus was recently identified as a key brain region in which the presence of TDP-43 pathology could accurately discriminate TDP-43 proteinopathy cases with clinical amyotrophic lateral sclerosis (ALS). The objective of the present study was to assess the hypoglossal nucleus in behavioral variant frontotemporal dementia (bvFTD), and determine whether TDP-43 in this region is associated with clinical ALS. Twenty-nine cases with neuropathological FTLD-TDP and clinical bvFTD that had not been previously assessed for hypoglossal TDP-43 pathology were included in this study. Of these 29 cases, 41% ($n = 12$) had a dual diagnosis of bvFTD-ALS at presentation, all 100% ($n = 12$) of which demonstrated hypoglossal TDP-43 pathology. Of the 59% ($n = 17$) cohort that presented with pure bvFTD, 35% ($n = 6$) were identified with hypoglossal TDP-43 pathology. Review of the case files of all pure bvFTD cases revealed evidence of possible or probable ALS in 5 of the 6 hypoglossal-positive cases (83%) towards the end of disease, and this was absent from all cases without such pathology. In conclusion, the present study validates grading the presence of TDP-43 in the hypoglossal nucleus for the pathological identification of bvFTD cases with clinical ALS, and extends this to include the identification of cases with possible ALS at end-stage.

1. Introduction

The TAR DNA-binding protein 43 (TDP-43) in behavioral variant frontotemporal dementia (bvFTD) was recently proposed to have a regional distribution, initiated in the orbitofrontal cortex and amygdala before progressing to the frontal and temporal cortices, eventually involving the motor system, visual cortex and cerebellum [5]. Although the majority of bvFTD cases were found to have TDP-43 pathology in motor system regions, less than half of these demonstrated clinical features suggestive or diagnostic of amyotrophic lateral sclerosis (ALS) [5].

In our recent analysis, the hypoglossal nucleus emerged as a key brain region in which the presence of TDP-43 pathology could accurately discriminate '*TDP-43opathy*' cases with coexistent clinical features of ALS [20]. However, TDP-43 pathology was seen in the hypoglossal nucleus of 13% of the bvFTD cohort assessed [20]. Based on the premise that pathological deposition precedes clinical symptomology [4], the present study set out to determine if bvFTD cases with TDP-43 pathology in the hypoglossal nucleus had developed or were developing ALS by end-stage.

2. Material and methods

2.1. Case selection

Cases with bvFTD were selected from a neuropathological series of cases collected by the Sydney Brain Bank through regional brain donor programs. The brain donor programs hold approval from the Human Research Ethics Committees of The University of New South Wales, and comply with the statement on human experimentation issued by the National Health and Medical Research Council of Australia. All cases had previously undergone standardized detailed neuropathological characterization [8,14]. Patients with bvFTD were diagnosed during life by experienced clinicians using standard clinical diagnostic criteria [13,16] following a medical interview, cognitive testing and an

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informant history. Standardized tests were used to longitudinally follow patients and controls, with their last assessments performed within 14 months of death. Clinical data were ascertained from an integrated clinical and neuropathological database and by retrospective review of patient clinical files. This research project was approved by the Human Research Ethics Committee of the University of New South Wales. Cases with neuropathological FTLD-TDP and clinical bvFTD not previously analysed in [20] were included in this study ($n = 27$) as well as two bvFTD cases previously assessed that met clinical criteria for possible ALS by endstage ($n = 2/22$ from [20] Fig. 2c). Of these 29 bvFTD cases, 59% ($n = 17$) did not have a clinical diagnosis of possible or probable ALS at onset and are referred to here as 'pure bvFTD' while the others had both bvFTD and possible or probable ALS at onset ($n = 12$, 41%).

2.2. Analysis of TDP-43 pathology in the hypoglossal nucleus

Formalin-fixed, paraffin-embedded tissue blocks of the hypoglossal nucleus were sectioned at 10 μm and immunostained with the anti-phospho TDP-43 monoclonal antibody (1:80,000, TIP-PTD-M01, Cosmo Bio). All slides were counterstained with haematoxylin to visualize neurons and other cells. In cases identified to have TDP-43 cytoplasmic inclusions in the hypoglossal nucleus (Fig. 1), TDP-43 immunostaining was also performed on sections from the motor cortex and where available, the spinal cord. The severity of TDP-43 pathology in each section was graded on a four-point severity scale: 0 = no detectable pathology, 1 = mild pathology, 2 = moderate and 3 = frequent pathology. Assessments of TDP-43 pathology were performed by two raters blind to case details with an inter- and intra-rater variance of <5%.

2.3. Severity of upper and lower motor neuron loss

The severity of Betz cell loss in the motor cortex and motor neuron loss in the anterior horn of the spinal cord was graded semi-quantitatively using haematoxylin and eosin stained sections, an Olympus microscope at 100-200× magnification and a 5-point severity scale: 0 = no loss, 1 = mild loss, 2 = moderate loss, 3 = severe loss, 4 = complete loss. Assessments of cell loss were performed by two raters blind to case details with an inter- and intra-rater variance of <5%.

2.4. Genetic analyses

2.4.1. Frozen tissue

Frozen tissue was available in 75% ($n = 9$) of bvFTD-ALS and 76% ($n = 12$) of pure bvFTD cases, and was screened for genetic mutations (*C9ORF72* and *GRN*) using previously published methods [12,17].

2.4.2. P62 and dipeptide repeat proteins in cerebellum

Formalin-fixed, paraffin-embedded tissue blocks of the cerebellum cortex were available for 67% ($n = 8$) of bvFTD-ALS and 76% ($n = 13$) pure bvFTD cases, and were sectioned at 10 μm, and immunostained with antibodies to p62 (cat #610833, BD Biosciences, 1:500), phosphorylated TDP-43 (TIP-PTD-M01, Cosmo Bio, 1:80,000) and poly-GA (specificity detailed previously [15], courtesy of Prof M Hasegawa, 1:1500). All slides were counterstained with haematoxylin to visualize neurons, and assessments for P62 and dipeptide repeat protein inclusions characteristic of *C9ORF72* expansions [2,15] were performed at 200–400× magnification.

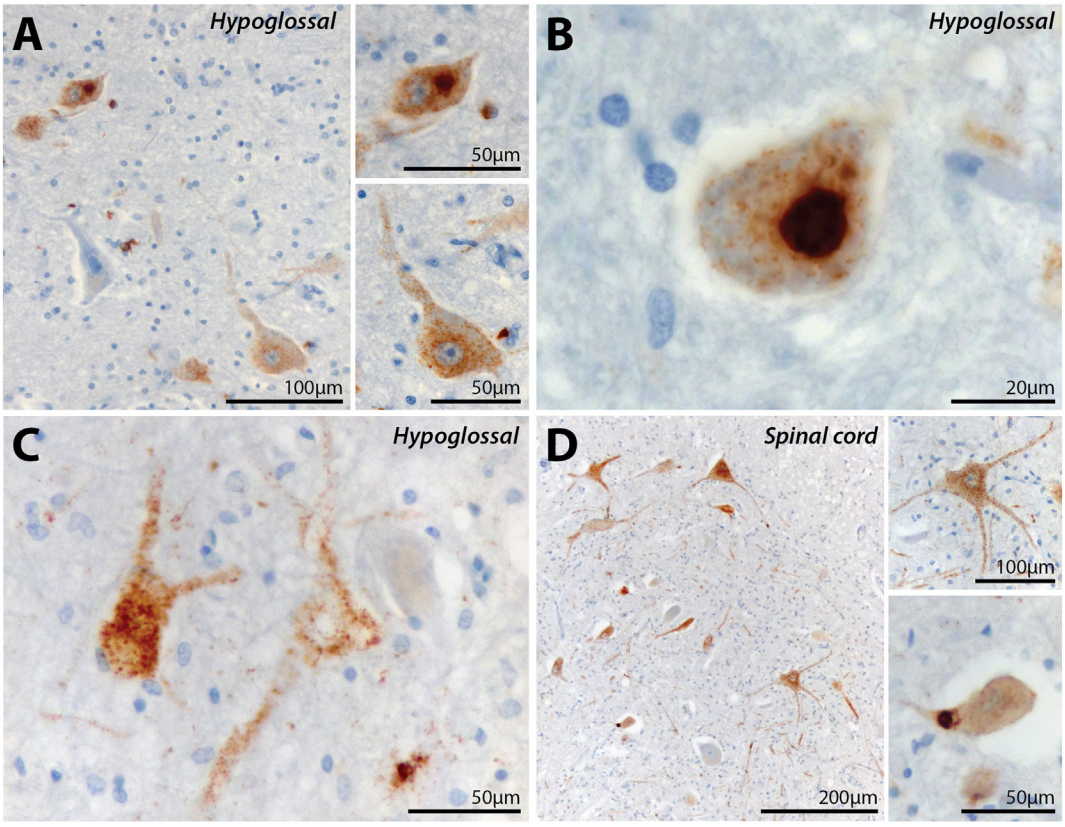

Fig. 1. TDP-43 in the hypoglossal nucleus and spinal cord of bvFTD cases. TDP-43 cytoplasmic inclusions (A top inset, case #1, B, case#4), punctate TDP-43 granules (A bottom inset, C) and TDP-43 glial inclusions (A) were identified in the hypoglossal nucleus of bvFTD cases. In one case (C, case #6), only punctate TDP-43 granules were present in the hypoglossal nucleus, and numerous TDP-43 granules with one cytoplasmic inclusion were observed in the spinal cord (D, case #6).