

## LETTERS

## C9ORF72 in Dementia with Lewy bodies

## INTRODUCTION

Recent studies have shown that a large hexanucleotide expansion in *C9ORF72* is the most common cause of inherited Frontotemporal Lobar Degeneration (FTLD) and Motor Neuron Disease (MND).<sup>1</sup> In pathological terms, expansion carriers show a distinctive molecular signature within the dentate gyrus granule cells and CA4 pyramidal cells of the hippocampus and granule cells of the cerebellum characterised by TDP-43-negative, but p62-positive, neuronal cytoplasmic inclusions (NCI).<sup>2</sup> Such inclusions contain dipeptide repeat proteins (DPR)<sup>2-3</sup> generated through non-ATG initiated translation of the expanded region of the gene.<sup>4-5</sup> On immunohistochemistry, an equivalent pattern of immunostaining is observed employing either antibodies to p62 or DPR, and that p62 immunostaining is an effective tool for identification of pathology associated with the presence of hexanucleotide expansions in *C9ORF72*.<sup>2-3</sup> In clinical terms, psychosis is one of the major clinical traits in patients with FTLD and/or MND who carry expansions in *C9ORF72*.<sup>6-8</sup> Given that psychosis is also common in Dementia with Lewy bodies (DLB), we previously genetically screened 102 patients with clinically diagnosed DLB and detected an expansion in *C9ORF72* in two patients.<sup>9</sup> Consequently, we immunostained tissue sections of hippocampus and cerebellum for p62 protein from a series of 53 pathologically confirmed cases of DLB in order to ascertain to what extent expansions in *C9ORF72* might be present in this disorder.

## METHODS

Brain tissues were available from a series of 53 patients with pathologically confirmed DLB within the Manchester Brain Bank. Three of these cases had been assessed in the previous genetic screen, though neither of the two cases in whom an expansion had been detected was represented in the autopsy cohort. All had been obtained with full ethical permission following consent by the next of kin. Paraffin sections were cut (at a thickness of 6 µm) from formalin fixed blocks of temporal cortex (with hippocampus) and cerebellar cortex and immunostained for p62 proteins (rabbit polyclonal antibody

to p62-lck ligand, B D Biosciences, Oxford, UK, 1:100 dilution), involving pressure cooking the sections for antigen retrieval and employing a standard ABC Elite kit (Vector, Burlingame, California, USA) with DAB as chromagen.<sup>2</sup> Immunostained sections were assessed for presence of p62-immunoreactive NCI within the dentate gyrus and CA2/3/4 regions of the hippocampus, and within the granule cells of the cerebellum.<sup>2</sup>

## RESULTS

No p62-immunoreactive NCI were seen within the hippocampus or cerebellum in any of the 53 cases.

## DISCUSSION

Previously, when screening a series of 102 patients fulfilling criteria for probable DLB we detected an expansion in *C9ORF72* in two patients.<sup>9</sup> Similar to expansion carriers with FTLD<sup>6-7</sup> or MND,<sup>8</sup> both patients displayed psychotic features, though in neither was a previous family history of dementia recorded, nor was there pathological confirmation of DLB or other underlying neurodegenerative disease. Therefore, it remains possible that these two individuals were misdiagnosed. Indeed, a frontotemporal dementia phenotype that mimics DLB has been reported.<sup>10</sup> We therefore investigated 53 pathologically confirmed cases of DLB for an expansion in *C9ORF72*, using the presence of p62-immunoreactive NCI in hippocampus and cerebellum as a surrogate marker, but did not detect any cases where relevant tissue changes were present. Although only three of the cases had undergone formal genetic analysis for an expansion in *C9ORF72* and had been shown to be negative, evidence indicates that the presence of p62-positive NCI in these brain regions, like the presence of DPR, can nevertheless act robustly as a marker of the expansion in the absence of genetic analysis.<sup>2-3</sup> From the present study, we therefore conclude that expansions in *C9ORF72* in pathologically confirmed cases of DLB are unlikely, and in those patients bearing expansions in clinically assessed cohorts, an atypical presentation of an underlying process of FTLD is likely to be present. Nonetheless, Cooper-Knock and colleagues have reported one of 377 pathologically confirmed patients with Parkinson's disease to bear an expansion in *C9ORF72*, and one of 17 other patients with an expansion in *C9ORF72* to show pathological changes in synuclein, suggesting that overlaps between an expansion in *C9ORF72* and

synucleinopathies can, if infrequently, indeed occur.<sup>11</sup>

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**Contributors** DMAM conceived the study, performed microscopic analyses and prepared the manuscript. AR and YD prepared tissue sections and performed immunohistochemical staining. JSS assisted with clinical characterisation of the cohort.

**Competing interests** None.

**Ethics approval** Ethics approval was provided by Newcastle and North Tyneside 1 Local Research Ethics Committee under Generic Tissue Bank Ethical Agreement.

**Provenance and peer review** Not commissioned; externally peer reviewed.



**To cite** Robinson A, Davidson Y, Snowden J S, et al. *J Neurol Neurosurg Psychiatry* 2014;**85**:1435–1436.

Received 10 January 2014

Revised 4 March 2014

Accepted 5 March 2014

Published Online First 19 March 2014

*J Neurol Neurosurg Psychiatry* 2014;**85**:1435–1436.  
doi:10.1136/jnnp-2014-307622

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