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Research Report

Expression of collagen XVII and ubiquitin-binding protein p62 in motor neuron disease

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ABSTRACT

Collagen involvement in motor neuron disease has been suggested by several earlier studies. Recently, we found collagen XVII to be expressed in locations in the human brain that include those damaged in motor neuron disease. In this study, we examined the extent of motor neuron disease-related changes in the brain of 9 subjects using ubiquitin-binding protein p62/sequestosome 1 (p62) immunohistochemistry. We then assessed whether or not the expression of collagen XVII was altered in relation to the p62 immunoreactive lesions. We found that neuronal collagen XVII expression in motor neuron disease remains similar to that seen in the normal human brain and thus a change in collagen XVII expression is not an immunohistochemically detectable feature of motor neuron disease. We also found that the regional distribution of p62 varied according to clinical presentation: p62 immunoreactive inclusions were found in the frontal cortex, hippocampus and cerebellum only in subjects with a history of psychiatric morbidity. Our study supports the re-definition of motor neuron disease as a multisystem disorder with a wide clinicopathological spectrum, and we advocate addressing psychiatric symptomology in future studies of motor neuron disease.

1. Introduction

Several studies have pointed towards cutaneous abnormalities in the motor neuron disease (MND) amyotrophic lateral sclerosis (ALS) (Ono et al., 1986, 1990; Ono and Yamauchi, 1992, Provinciali et al., 1994; Kolde et al., 1996; Ono et al., 1998). More specifically, an association between the blistering skin disease, bullous pemphigoid, in which collagen XVII (coll XVII) is targeted by autoantibodies, and ALS has been suggested (Chosidow et al., 2000). Recently, we found coll XVII to be

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Abbreviations: ALS, amyotrophic lateral sclerosis; CNS, central nervous system; coll XVII, collagen XVII, FTLD-U/ALS, frontotemporal lobar degeneration with ubiquitinated inclusions and amyotrophic lateral sclerosis, IHC, immunohistochemistry; IR, immunoreactive; MND, motor neuron disease; NCI, neuronal cytoplasmic inclusions; NT, neurites; p62, ubiquitin-binding protein p62/sequestosome 1; PMA, progressive muscular atrophy; TDP-43, trans-activating responsive sequence DNA-binding protein 43

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expressed in neurons in the human brain, including the motor neurons of the neocortex, brain stem and spinal cord (Seppänen et al., 2006, 2007), which in MND are typically lost or display inclusion bodies and cytoskeletal abnormalities (Xiao et al., 2006). The function of coll XVII in neurons is unclear, but in the skin, where it is abundantly expressed at the dermal–epidermal junction, coll XVII is involved in providing stable adhesion of the cytoskeleton to the extracellular matrix (Franzke et al., 2005).

In MND, by definition, upper or lower motor neuron lesions with corticospinal tract degeneration are seen. In addition, distinctive neuronal cytoplasmic inclusions (NCI) are readily visualized in both sporadic and familial cases of MND by applying ubiquitin immunohistochemistry (IHC). Likewise, the ubiquitin-binding protein p62/sequestosome 1 (p62) has been shown to be a common component of NCIs in neurodegenerative disorders including MND (Table 1, Kuusisto et al., 2008). Interestingly, it has recently been reported that trans-activating responsive sequence DNA-binding protein 43 (TDP-43)- and p62-immunoreactive (IR) pathology is seen in MND not only in the pyramidal motor system but more widely in the central nervous system (CNS) (Geser et al., 2008; Hiji et al., 2008).

The objectives of this study were to assess the distribution of p62-immunoreactivity in MND and to determine whether or not the expression of coll XVII is altered in association with the p62-IR lesions.

2. Results

The results of the p62- and coll XVII-stainings are given in Table 2 and Fig. 1. The expression of coll XVII in MND cases was comparable with that seen in the unaffected subjects previously described by us when using the same antibody and detection system (Seppänen et al., 2007). The intraneuronal staining of collagen XVII was widely dispersed in the neuronal cytoplasm, whereas the p62 staining was sharp and demarcated to the NCIs and neurites (NT) (Fig. 1). Nonparametric Spearman's correlation tests displayed no correlation between the expression of coll XVII and the quantity of p62-positive NTs or NCIs.

p62-IR pathological lesions were seen in all 9 subjects, though the anatomical distribution varied. However, in the motor cortex, at the level of the medulla in the nucleus

Table 1 – Published reports on p62-immunoreactivity in subjects with clinically and histologically diagnosed motor neuron disorders

Authors and publication year	Cases studied, terminology as given in the publication	Anatomical regions evaluated	Results	p62 antibodies used as given by the authors
Arai et al., 2003	1 FTD/MND	Cerebral cortex hippocampus temporal white matter	p62-immunoreactive NCIs neuronal and dystrophic NTs, white matter inclusions, p62 immunoreactivity in C4d-positive oligodendroglia, p62-immunoreactivity in astrocytes	Polyclonal p62-N, Progen, Heidelberg, Germany Polyclonal p62-C, Progen
Furukawa et al., 2004	6 FTD/MND 2 aPiD/MN symptoms	Cerebral cortex dentate gyrus	p62- and ubiquitin immunoreactive NCIs and NTs	Polyclonal GP62-N, Progen
Mizuno et al., 2006	24 ALS 2 ALS/dementia 2 ALS/basophilic inclusions	Lumbar spinal cord hippocampus	p62-immunoreactive NCIs in anterior horn cells and dentate gyrus in ALS/dementia cases	Polyclonal GP62-N, Progen
Parkinson et al., 2006	1 PMA	Motor cortex nucleus XII spinal cord	p62-immunoreactive lower motor neuron NCIs and NTs, coiled body inclusions in oligodendroglia in motor cortex	Polyclonal p62/sequestome 1, Progen
Seelaar et al., 2007	4 familial FTD/MND	Cerebral cortex hippocampus striatum thalamus substantia nigra locus coeruleus pons medulla cerebellum spinal cord	p62-, ubiquitin and TDP-43 immunoreactive NCIs, p62 and TDP-43 immunoreactive and ubiquitin negative glial inclusions	p62, Biosciences Pharmingen, San Jose, CA, USA
Hiji et al., 2008	5 ALS 5 FTLD/MND 5 p-FTLD/MND	White matter	p62-immunoreactive NCIs throughout white matter of FTLD/MND cases. Incomplete co-localization with TDP-43, varying according to clinical presentation	Polyclonal p62-C, Progen

FTD = frontotemporal dementia, MND = motor neuron disease, NCI = neuronal cytoplasmic inclusion, NT = neurite, aPiD = atypical Pick`s disease, ALS = amyotrophic lateral sclerosis, PMA = progressive muscular atrophy, FTLD = frontotemporal lobar degeneration, p-FTLD/MND = pathologically compatible with FTLD/MND but no cognitive decline.