

## Negative results

## *FUS* is not dysregulated by the spinal bulbar muscular atrophy androgen receptor polyglutamine repeat expansion

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## ABSTRACT

Spinal bulbar muscular atrophy (SBMA) and amyotrophic lateral sclerosis are two distinct forms of motor neuron disease with different genetic causes, pathology, and clinical course. However, both disorders are characterized by the progressive loss of lower motor neurons and by a similar protective response to growth factors in animal models, therefore raising the possibility of an overlap in the final pathogenic cascade. Mutations in the *FUS* gene and fused in sarcoma (FUS) protein pathology have now been identified in some amyotrophic lateral sclerosis cases, while a CAG expansion in the androgen receptor gene is known to cause SBMA. Recently, multiple lines of evidence have identified *FUS* as a major target of the androgen receptor, suggesting that *FUS* could be dysregulated in SBMA motor neurons. We have investigated this possibility by using a well-established mouse model of SBMA and our analysis of primary motor neuron cultures, spinal cords, and microdissected motor neurons show no evidence for *FUS* dysregulation.

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### 1. Introduction

Mutations in Fused in Sarcoma (*FUS*) have been found to cause up to 4% of familial amyotrophic lateral sclerosis (Ferraiuolo et al., 2011). Spinal bulbar muscular atrophy (SBMA) is a slowly progressive adult-onset motor neuron (MN) disease characterized by the loss of lower MNs (LMNs) in the spinal cord and brain stem. It is caused by a CAG repeat expansion in the first exon of the androgen receptor (AR) gene which results in an extended polyglutamine tract (AR SBMA poly-Q) (Rhodes et al., 2009). The AR is a ligand-dependent transcription factor and the AR SBMA poly-Q expansion has been shown to cause disease through a variety of mechanisms, including an amplification of native AR interactions and function (Nedelsky et al., 2010).

Amyotrophic lateral sclerosis and SBMA fall under the general class of MN diseases, but have different genetic causes, pathology, and clinical course. Nonetheless, both diseases are associated by the

progressive adult onset loss of LMNs, similarities in specific pathology findings, and a positive response of animal models to general protective treatments (Forsberg et al., 2012; Papanikolaou and Ellerby, 2009; Sopher et al., 2004). These findings suggest the possibility that the diseases share features in the pathogenetic cascade that leads to neuronal death (Papanikolaou and Ellerby, 2009).

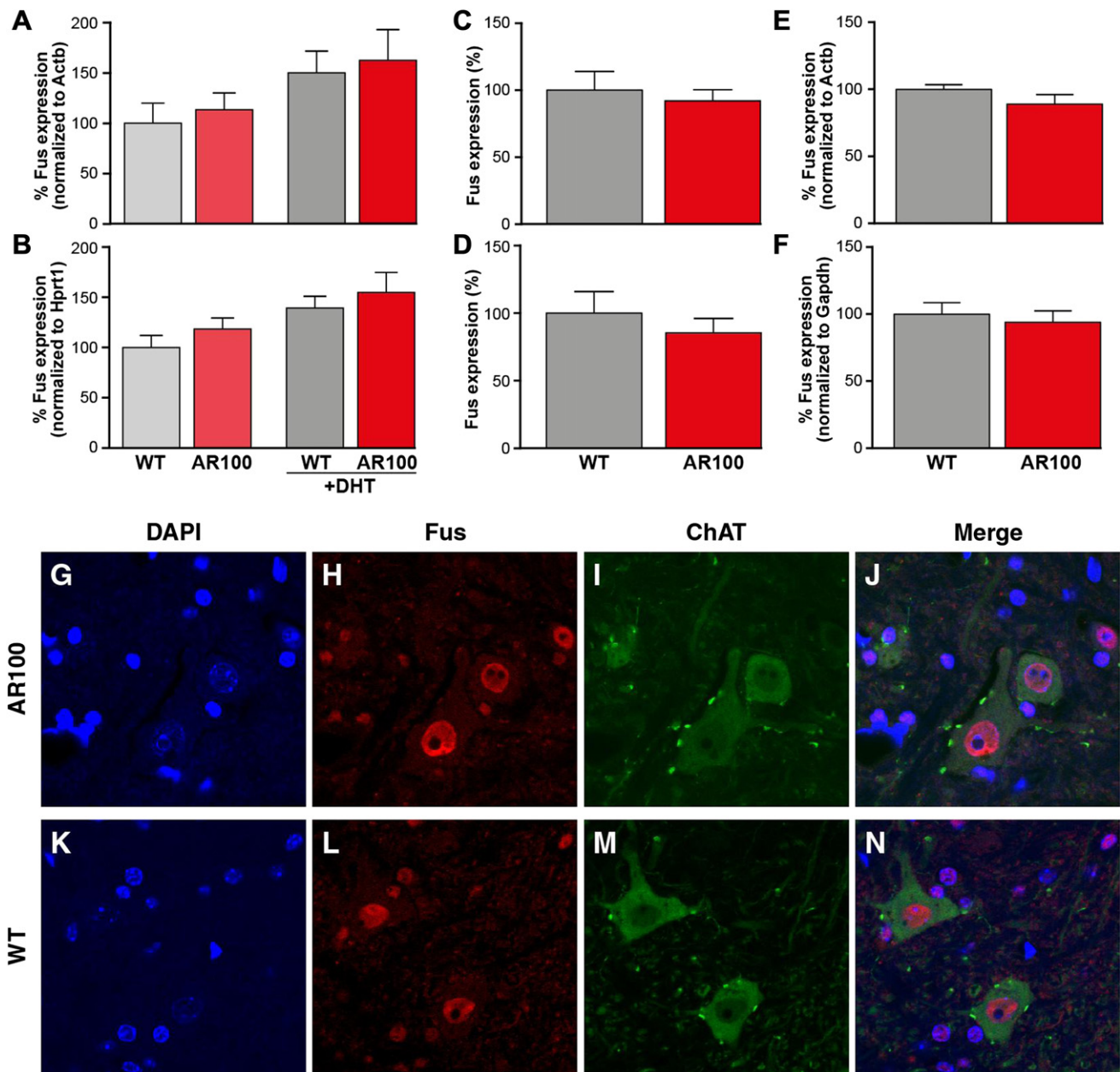
Two recent independent studies, focusing on prostate cancer cells, have shown that fused in sarcoma (*FUS*) levels are regulated by the AR in an androgen-dependent fashion (Brooke et al., 2011; Haile et al., 2011). These results raise the possibility that *FUS* might be misregulated in SBMA, and this might contribute to the MN degeneration in the disease, offering an intriguing link between these 2 distinct forms of MN degeneration. Here we investigate this possible link by determining if *FUS* expression is dysregulated in a well characterized SBMA mouse model (AR100) carrying a 100 poly-Q expanded mutation in the AR (Sopher et al., 2004).

### 2. Methods

Details of mice, embryonic MN culture, laser capture microdissection, real-time quantitative polymerase chain reaction, Western

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**Fig. 1.** *FUS* is normally expressed in the presence of AR100 repeat expansion. *FUS* messenger RNA (mRNA) levels in embryonic motor neurons, normalized to *Actb* (A) and *Hprt1* (B). No significant (ns) change is present between AR100 and wild type (WT). A significant increase in *FUS* levels is present after dihydrotestosterone (DHT) treatment using 2-way analysis of variance [ $F(1,8) = 10.108$ ;  $p = 0.013$ ]. (C) Protein levels in 3-month-old AR100 ( $n = 6$ ) and littermate control (WT) ( $n = 5$ ) spinal cords normalized to  $\beta$ -actin (ns, by 2-tailed unpaired  $t$  test;  $p = 0.62$ ). (D) Protein levels in 12-month-old AR100 ( $n = 3$ ) and WT ( $n = 3$ ) spinal cords normalized to  $\beta$ -actin (ns, by 2-tailed unpaired  $t$  test;  $p = 0.49$ ). *FUS* mRNA expression in laser capture microdissection motor neurons (MNs) of AR100 and WT mice, normalized to *Actb* (E) and *Gapdh* (F) (ns, by 2-tailed unpaired  $t$  test;  $p = 0.30$ ,  $p = 0.63$ , respectively). Fused in sarcoma protein (Fus) localization is normal in 18-month-old AR100 mice MNs. Confocal images from lumbar spinal cord sections of WT (G–J) and AR100 (K–N) mice. Choline acetyltransferase (ChAT) (green), Fus (red), DAPI (blue). Fus appears to localize mainly in the nucleus of MNs and other cell types in both genotypes.

blot, and immunohistochemistry methods are given in the Supplementary data.

### 3. Results

To assess the effect of the AR SBMA poly-Q repeat expansion and dihydrotestosterone (DHT) on *FUS* regulation in MNs, we initially determined *FUS* messenger RNA (mRNA) levels in purified embryonic MN (eMN) cultures obtained from AR100 and wild type (WT) littermate embryos. Taqman analysis showed that DHT treatment increases *FUS* levels [ $F(1,8) = 10.108$ ;  $p = 0.013$ ], but there was no significant difference

between AR100 and WT eMNs in both DHT-treated and untreated conditions. (Fig. 1A and B).

To assess if the presence of the AR SBMA poly-Q repeat expansion caused a change in *FUS* levels in the context of the mature adult central nervous system, we tested *FUS* protein levels in spinal cord lysates of presymptomatic 3-month-old mice. The results showed no significant difference in *FUS* protein levels between AR100 mice and littermate WT controls (Fig. 1C).

*FUS* protein levels in the spinal cord come from an extremely diverse population of cells and this might mask possible differences present in specific cell types. We therefore analyzed *FUS* mRNA

levels specifically in LMNs isolated by laser capture microdissection. However, no significant changes were observed in AR100 LMNs (Fig. 1E and F).

Finally, to test if *FUS* dysregulation plays a role during the active disease process, we analyzed *FUS* protein levels in spinal cords of aged AR100 and control mice and found no significant differences (Fig. 1D). We also investigated *FUS* localization in lumbar spinal cord sections from end-stage AR100 and control mice. The staining revealed that *FUS* was localized in the nucleus throughout cells in the spinal cord and also specifically in MNs, in both genotypes (Fig. 1G–N).

#### 4. Discussion

*FUS*, which when mutated can be causative of amyotrophic lateral sclerosis and is found to mislocalize in patients' spinal cords (Ferraiuolo et al., 2011), is known to interact with nuclear hormone receptors and the AR (Haile et al., 2011; Powers et al., 1998). *FUS* is regulated at both the protein and mRNA levels through the AR in response to androgen stimuli (Brooke et al., 2011; Haile et al., 2011). These findings, along with the recent evidence that the mutant expanded poly-Q AR exerts part of its pathogenicity through misappropriation of native function (Nedelsky et al., 2010), have raised the question as to whether *FUS* could be misregulated in SBMA.

Our results show that *FUS* mRNA levels are increased in response to DHT in both AR100 and control purified eMNs, but no difference between AR100 and controls was found. We then analyzed if any pathologic changes occurred in vivo and, to study the direct effect of the poly-Q expanded AR, and not secondary disease changes, we chose to conduct our initial analysis at the presymptomatic age of 3 months. Both *FUS* protein levels in spinal cord lysates and *FUS* mRNA levels in microdissected MNs showed no changes between AR100 and controls. Finally, we investigated the involvement of *FUS* in the active phases of disease by testing mice at advanced and end-stage time points. However, even at these symptomatic stages of disease, protein levels were unchanged and *FUS* localization was also normal.

In conclusion, our results show that *FUS* expression is not altered in the spinal cord or in MNs in AR100 mice and is therefore not influenced by the presence of the poly-Q expanded AR or by secondary changes occurring in active SBMA.

#### Disclosure statement

The authors declare no actual or potential conflicts of interest.

Mice were housed in controlled conditions in accordance with guidance issued by the Medical Research Council in Responsibility in the Use of Animals for Medical Research (1993) and all experi-

ments were carried out under License from the UK Home Office and with Local Ethical Review panel approval.

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#### Appendix A. Supplementary data

Supplementary data associated with this article and an extended version of the Introduction, Results, Methods, Discussion and References can be found, in the online version, at <http://dx.doi.org/10.1016/j.neurobiolaging.2012.09.008>.

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