

## Letter to the Editor, Brain

### TDP-43 mutations increase HNRNP A1-7B through gain of splicing function

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In a recent investigation Deshaies and colleagues identify HNRNP A1-7B, an isoform of the RNA binding protein (RBP) HNRNP A1, as present in *post mortem* amyotrophic lateral sclerosis (ALS) spinal motor neuron inclusions (Deshaies *et al.*, 2018). HNRNP A1 is an important player in ALS, as mutations in its low complexity domain (LCD) can be causative for the disease (Kim *et al.*, 2013). Intriguingly, the isoform described by Deshaies *et al.* includes exon 7B, which produces an extension of the LCD by 52 amino acids, pointing to a link between this alternative splicing event and neuronal protein aggregation. The authors further suggest that loss of RBP TDP-43 can influence this splicing event and result in an increase of the aggregation-prone 7B isoform. TDP-43 is abnormally mislocalised in >95% of ALS cases and when mutated can cause ALS (Harrison and Shorter, 2017).

TDP-43 binds RNA, preferentially at UG repeats, and is involved in alternative splicing (Buratti and Baralle, 2001). Importantly, its activity is extremely dosage sensitive, making physiological expression an essential condition for studying the effect of TDP-43 mutations on splicing. Recently, we and others have published an allelic series of novel physiological TDP-43 mouse mutant models and shown that C-terminal mutations induce RNA splicing gain of function (Fratta *et al.*, 2018; White *et al.*, 2018). In order to investigate the link between TDP-43, its mutations and *Hnrnpa1* exon 7B splicing, we utilised recently published

high quality iCLIP (Tollervey *et al.*, 2011; Rogelj *et al.*, 2012) and RNA-seq datasets (Kapeli *et al.*, 2016; Fratta *et al.*, 2018; White *et al.*, 2018) from human and mouse.

Deshaies *et al.* show by immunoprecipitation of TDP-43 followed by RT-PCR that TDP-43 directly interacts with *HNRNPA1* transcripts. However, their approach does not allow identification of the exact site of interaction (Deshaies *et al.*, 2018). To determine the exact binding sites, we pooled available mouse and human iCLIP data for TDP-43, which identifies the binding sites of TDP-43 on transcripts with single nucleotide resolution. We found that TDP-43 does not bind to exon 7B or its flanking introns, where UG rich motifs are present, but instead binds to *HNRNPA1* exon 8 (in human) and has very strong conserved binding sites within the 3'UTR of *HNRNPA1* in both human and mouse (**Figure 1A**).

In order to investigate the effect of TDP-43 loss of function in mouse CNS, we used two RNA-seq datasets where:

(1) TDP-43 knockdown through antisense oligonucleotides reduces levels of TDP-43 in the adult mouse brain (TDP-43 ASO-KD) (Polymenidou *et al.*, 2011);

(2) point mutation in TDP-43 RNA recognition motif 2 (RRM2) reduces its RNA binding capacity and therefore uncovers direct consequences of diminished TDP-43 binding to RNA (TDP-43 *RRM2mut*) (Fratta *et al.*, 2018).

Strikingly, both TDP-43 ASO-KD and TDP-43 *RRM2mut* displayed greater *skipping* of *Hnrnpa1* exon 7B (**Figure 1B**), rather than the greater *inclusion* levels described by Deshaies *et al.*

We then analysed the impact of C-terminal mutations in TDP-43 upon *Hnrnpa1* and its splicing. One mutation, TDP-43 Q331K, is causative for ALS in patients and has been shown to induce cognitive phenotypes in mice; the second mutation, TDP-43 *LCDmut*, has not been described in patients but lies within the hotspot for human TDP-43 ALS mutations and induces an ALS-like phenotype in mice (Fratta *et al.*, 2018; White *et al.*, 2018). Recent work on both lines has demonstrated a gain of splicing function effect of C-terminal TDP-43 mutants. Intriguingly, both mutations show a dose-dependent increase in *Hnrnpa1* exon 7B inclusion (**Figure 1B**), the opposite of what occurs in TDP-43 *RRM2mut* and TDP-43 ASO-KD. Both TDP-43 *LCDmut* and Q331K models, which show increased *Hnrnpa1* exon 7B inclusion, develop a clear neurodegenerative-disease phenotype (Fratta *et al.*, 2018; White *et al.*, 2018). This link agrees with Deshaies *et al.*'s finding that HNRNP A1-7B accumulates in ALS patient spinal cord motor neurons. Interestingly, in both TDP-43 *LCDmut* and the Q331K models, the increased exon 7B inclusion is detectable at all timepoints, but is more pronounced at late disease stages.

Finally, we examined whether the effects of changes in TDP-43 splicing activity on *HNRNPA1* exon 7B inclusion were conserved in humans. We analysed RNA-seq data from shRNA-mediated TDP-43 knockdown in motor neurons generated from wildtype human iPSCs (iMNs) (Kapeli *et al.*, 2016). In accordance with results from our mouse model analysis, loss of TDP-43 in human settings also resulted in substantial reduction in exon 7B inclusion (**Figure 1C, D**).

In summary, our data here support the direct involvement of TDP-43 in *HNRNPA1* splicing. We show that TDP-43 gain of function, but not loss of function, leads to the increased inclusion of exon 7B. Although our findings may differ from Deshaies *et al* on how TDP-43 loss of function impacts on *HNRNPA1* splicing, both our data and Deshaies *et al* support a link between TDP-43, the HNRNP A1-7B isoform and ALS.

## References

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**Figure 1 HNRNP A1 isoforms in TDP-43 loss- and gain of function models. (A)**

Annotated *Hnrnpa1* transcripts alongside iCLIP clusters indicating regions of TDP-43 binding in mouse (left) and human (right). Exon 7B highlighted in red. **(B)** Percentage Spliced In (PSI) values for *Hnrnpa1* exon 7B in six published RNA-seq datasets: TDP-43 ASO-KD, *RRM2mut* embryonic day 14.5 frontal cortex, *LCDmut* embryonic day 14.5 head, *LCDmut* 6-month spinal cord, Q331K 5-month spinal cord and Q331K 20-month spinal cord, \*  $p < 0.05$ , \*\*  $p < 0.01$ . **(C)** Representative RNA-seq read traces with sashimi plots for *HNRNPA1* in WT and TDP-43 KD human iMNs. Proportional levels of exon 7B inclusion (top) and skipping (bottom) labelled in each trace. Exon 7B highlighted in red on annotated *HNRNPA1* transcripts. **(D)** PSI values for *HNRNPA1* exon 7B in TDP-43 KD human iMN RNA-seq.

