RESEARCH LETTER

No Effect on SOD1 Splicing by TARDBP or FUS Mutations

Amyotrophic lateral sclerosis (ALS) is characterized by a neuronal loss in the motor cortex, brainstem, and spinal cord that progresses over a 3 to 5 year period. Approximately 90% of cases are considered sporadic, while the other 10% of cases are familial.\(^1\) Extensive research has been conducted on the SOD1 (RefSeq NM_000454.4) gene after mutations were identified more than 17 years ago in 15% to 20% of familial ALS cases, as well as a small number of sporadic ALS cases in later reports.\(^2\) In the last 2 years, mutations in TARDBP, which encodes TAR DNA-binding protein 43 (TDP-43), and in FUS have been identified in both familial and sporadic ALS cases.\(^2\)\(^3\) Both genes encode for RNA/DNA binding proteins, are mainly localized in the nucleus, and are implicated in the regulation of RNA processing.\(^3\) Specifically, TDP-43 and FUS have been shown to be associated with other splicing factors and are believed to play a role in splicing regulation, as variation in their expression level influences the splicing of certain targets.\(^5\) Interestingly, mutant TDP-43 proteins identified in patients with ALS were recently reported to be more stable than wild-type TDP-43 and to display an enhanced interaction with mutant TDP-43, which display an increased half-life compared with wild-type TDP-43. The authors concluded on a note about the efforts needed to determine whether the increased association affects the RNA targets for TDP-43, FUS/translocated in liposarcoma protein, or both.\(^6\) Considering that abnormal RNA processing and splicing patterns are involved in neurodegenerative diseases\(^1\) and that variation in the RNA splicing of SOD1 can cause familial ALS by destabilizing the resulting protein,\(^1\) our aim was to assess if TARDBP and/or FUS ALS-predisposing mutations that are known to lead to the accumulation of TDP-43 or FUS aggregations in the cytoplasm also lead to aberrant SOD1 RNA splicing events, and to determine if SOD1 could be an RNA target for TDP-43, FUS, or both.

Methods. We studied total RNA prepared from immortalized lymphoblastoid cells of 1 healthy control individual as well as 7 participants with different TARDBP mutations (p.D169G, p.G287S, p.G348C, p.R361S, p.Y374X, p.A382T, p.N390D) and 4 participants with different FUS mutations (p.P18S, p.G287S, p.G348C, p.Y374X) and the Moloney murine leukemia virus reverse transcriptase enzyme (Invitrogen, Carlsbad, California) per the manufacturer’s instructions. The amplifications were performed using 2 sets of primer pairs that covered the entire messenger RNA of SOD1. Polymerase chain reaction products were sequenced at the Genome Quebec Innovation Centre (Montreal, Quebec, Canada) using a 3730XL DNA analyzer (Applied Biosystems, Carlsbad, California). The first set amplified a region of 423 base pairs (bp) (c.398-462), starting at the beginning of exon 4. The second set of primers amplified a product of 528 bp (c.398-462), starting at the beginning of exon 4 and ending at the end of the 3’ untranslated region.

Results. Agarose gel electrophoresis showed that only 1 product was amplified for each of the complement DNA amplified (Figure). In addition, no variation was found in the sequence traces of the SOD1 complement DNA products.

Comment. We can conclude that TARDBP or FUS ALS-predisposing mutations do not affect the splicing of SOD1 and that, while it can’t be excluded that there may be a common ALS pathogenic pathway, it appears that mutant TARDBP and FUS do not act by affecting the splicing of the most frequently mutated gene, SOD1, in ALS.

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Figure. Agarose gel electrophoresis of SOD1 messenger RNA. The first amplified product of 423 base pairs (bp) for the control and the 11 TARDBP or FUS mutants is shown on top. The second product of 528 bp is shown under the first product for the same samples.
The article by Goldstein et al. is interesting; however, there are certain areas of concern.

In Table 4, the authors highlighted the intracerebral hematoma (ICH) size, further expansion in size, and outcome. Of 20 patients, 6 had an ICH of less than 10 mL and one, 13.7 mL. Only 2 of the 7 patients had an increase in hematoma size, and for 2 patients, no data on expansion was available. In the remaining 3 patients, ICH size decreased on repeated neuroimaging. Five of the 7 patients died, and 2 received rehabilitation. The size of the hematoma is an important predictor of death and prognosis in intracerebral hematoma. Any ICH with a volume less than 30 mL carries a better prognosis. Thus, significantly high mortality in patients with a small ICH volume is unusual. Any large-volume ICH carries high mortality and, in this study, all 7 patients with more than 100 mL of ICH died. The mortality rate in symptomatic ICH cases is 47%, but in this study it was 75%, which is high. The authors have not reported the cause of death in the patients with a small hematoma. Septicemia and gastrointestinal bleeding in patients with ICH is a significant cause of death. It would be interesting to know how many patients developed coagulopathies leading to bleeding from other sites, which may explain very high mortality.

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In reply

We would like to thank the authors for their comments on our article and appreciate having an opportunity to respond. First, Dr Pandey notes that hematoma size is an important predictor of death and prognosis, citing Christoforidis et al. The mortality rate in that study was 78% among those with symptomatic hemorrhagic transformation, similar to that of our cohort. However, their multivariable analysis did not examine whether hematoma volume independently predicted death; rather, they showed that a hematoma volume greater than 25 mL was an independent predictor of change in the National Institutes of Health Stroke Scale score among survivors. Much of the literature on ICH volume and outcome is based on patients with symptomatic hemorrhagic transformation, and it may be that the size of the original infarct is as important as ICH volume. In fact, for patients with small hemorrhage volumes, infarct size may be an even more powerful predictor of outcome than ICH volume.

Second, Dr Pandey comments that the mortality rate of symptomatic ICH is expected to be lower than in our cohort, at 47%; this appears to reflect the mortality rate from the original National Institute of Neurological Disorders and Stroke study. Given the small numbers involved, this represents a difference of only a few patients surviving vs dying, and we suspect that this difference is owing to chance. Patients with symptomatic ICH had disproportionately large strokes (perhaps as expected) that may have contributed to the poor out-