**Supplementary data**

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5. **Table e-1**

**Table e-1.** Oligonucleotide sequences used for this study.

|  |  |  |
| --- | --- | --- |
| Name | Sequence (5′-3) | Application |
| *SQSTM1* Fwd | TCCATCCTGGGAGACAGAAAC | PCR |
| *SQSTM1* Rev | GCCTCATTGCCAAGAAACTAT | PCR |
| *SQSTM1* Exon 4-7 Fwd | CTGTGGTAGGAACCCGCTAC | RT-PCR |
| *SQSTM1* Exon 4-7 Rev | GAGGAAGATCGCCTTGGAGT | RT-PCR |
| *GAPDH* Fwd | GAAGATGGTGATGGGATTTC | RT-PCR |
| *GAPDH* Rev | GAAGGTGAAGGTCGGAGTC | RT-PCR |

**2. Table e-2**

**Table e-2. Mutations present within the fALS cohort**

|  |  |  |
| --- | --- | --- |
| Gene | Mutation | Frequency (*n*) |
| *C9orf72* | >23 CCGGGG | 10 |
| *SOD1* | Ala5>Val | 66 |
| *SOD1* | Ala5>Thr | 7 |
| *SOD1* | Gly13>Arg | 1 |
| *SOD1* | Val15>Met | 6 |
| *SOD1* | Gly17>Ala | 2 |
| *SOD1* | Gly38>Arg | 2 |
| *SOD1* | Gly42>Asp | 4 |
| *SOD1* | Gly42>Ser | 2 |
| *SOD1* | His49>Arg | 3 |
| *SOD1* | Leu85>Val | 1 |
| *SOD1* | Gly86>Arg | 12 |
| *SOD1* | Val88>Met | 1 |
| *SOD1* | Ala90>Val  | 2 |
| *SOD1* | Asp91>Ala | 2 |
| *SOD1* | Gly94>Ala | 6 |
| *SOD1* | Glu101>Gly | 14 |
| *SOD1* | Glu101>Lys | 10 |
| *SOD1* | Leu107>Val | 3 |
| *SOD1* | Ile114>Thr | 10 |
| *SOD1* | Glu134>Ala | 2 |
| *SOD1* | Leu145>Phe | 4 |
| *SOD1* | Val149>Gly | 5 |
| *SOD1* | 5D splice junction A>G 11 bp <E5 | 1 |
| *TDP43* | Ala90>Val | 1 |
| *TDP43* | Gly287>Ser | 1 |
| *TDP43* | Gly294>Val | 1 |
| *TDP43* | Gly298>Ser | 7 |
|  *TDP43* | Ala321>Tyr | 1 |
| *TDP43* | Asn325>Asx | 5 |
| *TDP43* | His352>Asx | 1 |
| *TDP43* | Asn378>Asp | 1 |
| *TDP43* | Asn390>Asp | 1 |

**3. e-Methods**

*SV selection*

The bioinformatics software evaluated and prioritized structural variants (SVs) likely to modulate expression regulation within *SQSTM1.1*The candidate variant was chosen due to its high potential impact score, created from 24 different properties, including variability (repeat number, size, simple sequence repeat slippage), synergy of consecutive variants, trait association, nearby regulatory elements, signal for transcription factor binding sites, region conserved among mammals and primates, local drop in conservation value, and intron size.1

*PCR amplification and Sanger sequencing*

Endpoint PCR reactions were prepared according to the manufacturer’s recommendations to a final volume of 25 µl, containing 0.1 µl Amplitaq Gold DNA polymerase (Thermo Fisher Scientific, MA, USA), and 50 ng of human genomic DNA. Thermocycling was carried out with the following conditions: 94°C for 6 min and 35 cycles of 94°C for 40 sec, 55°C for 30 sec, and 72°C for 1 min. PCR products were fractionated on a 12% native-PAGE. For amplicon sequence confirmation, purification was performed using Diffinity Rapid Tips (Diffinity Genomics, PA, USA) as per manufacturer’s instructions and sent to the Australian Genome Research Facility (AGRF, Perth Australia) for Sanger sequencing.

*Cell Culture*

Olfactory neurosphere derived cells (ONS) cells, established according to previously published protocols2 were a kind gift from Professor Alan Mackay-Sim at Griffith University (Brisbane, Australia). ONS cells were proliferated in DMEM/F12 containing 10% fetal bovine serum prior to cell pellets being harvested for RNA and DNA extraction.

*DNA isolation*

DNA was extracted from ONS cultures, using the PureLink Genomic DNA Mini Kit (Invitrogen, Melbourne, Australia) according to the manufacture’s protocol.

*RNA isolation*

RNA was extracted from ONS cultures using the MagMAX-96 Total RNA Isolation Kit, including a DNase treatment (Life Technologies, CA, USA), according to the manufacturer’s protocol.

*RT-PCR and densitometry*

RT-PCRs were preformed using the One-step Superscript III RT-PCR kit with Platinum Taq polymerase (Life Technologies, CA, USA) according to the manufacturer’s instructions. Thermocycling for exon 4-7 of *SQSTM1* was carried out with the following conditions: one cycle of 55°C for 30 min and 94°C for 2 min followed by 23 cycles of 94°C for 40 sec, 55°C for 30 sec, and 68°C for 1 min. Thermocycling for *GAPDH* was carried out with the following conditions: one cycle of 42°C for 15 min and 95°C for 3 min, followed by 24 cycles of 95°C for 10 sec, 57°C for 30 sec, and 72°C for 1 min. RT-PCR products were fractionated on a 2% agarose gel, with GAPDH used as a loading control. Densitometric analysis of gels was performed using ImageJ software (National Institutes of Health, MD, USA), with *SQSTM1* mRNA transcript signal standardized to each respective *GAPDH* signal.

1. **e-References**

1. Saul R, Lutz MW, Burns DK, Roses AD, Chiba-Falek O. The SSV Evaluation System: A Tool to Prioritize Short Structural Variants for Studies of Possible Regulatory and Causal Variants. Hum Mutat 2016;37:877–883.

2. Féron F, Perry C, Girard SD, Mackay-Sim A. Isolation of Adult Stem Cells from the Human Olfactory Mucosa. Methods Mol. Biol. 2013.