

Supplemental materials

Neuropathological analysis of an ALS patient carrying a SOD1 missense variant and a C9orf72 repeat expansion

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Supplemental methods

Ethical and legal authorizations

The patients were enrolled in Tissue Bank at the Pitié-Salpêtrière Hospital (Paris, France) or Neuro-CEB French brain bank. Autopsies were authorized according to French regulation and approval of the Ministry of Higher Education and Research (agreement AC-2013-1887). Autopsy and genetic analyses were carried out after written informed consent obtained from patients and/or family members. All experiments in this study were approved by the Medical Research Ethics Committee of “Assistance Publique Hôpitaux de Paris” (#DC-2013-2031-A75, date of approval: 2014/07/10, revised the 2019/10/11). The sponsor for the genetic analysis protocol was Inserm (Institut National de la Santé et de la Recherche Médicale), Paris, France.

Immunohistochemistry

Brain tissue samples were fixed post-mortem with formaldehyde and embedded in paraffin. Samples were cut into 3 µm-thick sections using an Eprelia HM340E microtome (Eprelia, Portsmouth, NH, USA). Sections were stained with hematoxylin-eosin and Luxol fast blue.

Immunostainings were performed after deparaffinization with Ventana Benchmark UltraPlus Roche Diagnostics® automated system (Roche Diagnostics, Tucson, AZ, USA). Primary antibodies used in this study included anti-TDP-43 (10782-2-AP, Proteintech), anti-phosphorylated TDP-43 pS409/410 (TIP-PTD-M01A, Cosmo Bio, LTD), anti-p62 (610833, BD Biosciences), anti-poly GA (MABN889, Sigma-Aldrich), anti-poly GP (24494-1-AP, Proteintech), anti-poly-GR (23978-1-AP, Proteintech), and anti-Cu/Zn-SOD (#07-403-I, Merck Millipore). Antibodies were targeted with a biotin-free detection system (Ventana Medical Systems Basic DAB Detection Kit 250-001). The pretreatment and the antibodies used are listed in Supplemental Table S1.

Genetic analysis

DNA was extracted from frontal cortical fresh frozen slices of the patient. The *C9orf72* repeat was analyzed using a repeat-primed PCR amplification completed by a classical fluorescent fragment length analysis allowing the detection of non-expanded *C9orf72* alleles, as previously described [20]. These analyses were repeated twice for the patient to ensure reproducibility of the results. In addition, whole-exome sequencing analysis (WES) was performed as previously detailed [49] to select variants with a minor allele frequency (MAF) <0.005% in dbSNP, Hapmap, 1000genome, Exome Variant Server and gnomAD databases in 32 ALS related genes (including *ALS2*, *ANG*, *ANXA11*, *CCNF*, *CHCHD10*, *DAO*, *DCTN1*, *DNAJC7*, *FIG4*, *FUS*, *GLE1*, *GLT8D1*, *HNRNPA1*, *HNRNPA2B1*, *KIF5A*, *MAPT*, *MATR3*, *NEK1*, *OPTN*, *PFN1*, *SETX*, *SIGMAR1*, *SOD1*, *SQSTM1*, *SS18L1*, *TARDBP*, *TBK1*, *TIA1*, *TUBA4A*, *UBQLN2*, *VAPB* and *VCP*). Variants were validated using Sanger analysis with BigDye chemistry as recommended by the supplier (Applied Biosystems) and analyzed using SeqScape v2.5.

Construction of plasmids expressing WT or mutant SOD1 and cellular analysis

Plasmids expressing normal and mutant forms of SOD1 were established from cDNA amplified from SOD1-related patient spinal cord. First, SOD1 complete cDNA was amplified as previously described [42]. A second SOD1 fragment flanked by NotI and BglII restriction enzyme sites was further obtained using cloning primers (Supplemental Table S3), purified by phenol/chloroform extraction followed by ethanol precipitation with ammonium acetate and digested overnight by NotI and BglII restriction enzymes. These cohesive SOD1 fragments were ligated using T4 DNA ligase in the pMAPT^{WT} plasmid [37] digested with NotI and BglII (to remove the mcherry-MAPT transgene) and dephosphorylated with FastAP thermosensitive Alkaline Phosphatase. Ligation products were transformed in the E. coli One Shot TOP10 chemically competent cell line. DNA

extracted from clones were sequenced using cloning primers to check the resulting SOD1 amino acid sequence and select clones to be transfected. Plasmid DNA were prepared using PureLink™ HiPure Plasmid Maxiprep kit. All enzymes for cloning procedure were from (ThermoFisher Scientific).

For calcium phosphate transfection procedure, human HEK293 embryonic kidney cells were seeded at 25,000/well on polyornithine-coated glass coverslips in 24-well-plates. The day after, each well was subjected during 3 hours to the DNA–calcium phosphate co-precipitates obtained from mixing 1 µg of plasmid in HEPES-buffered saline containing sodium phosphate with a CaCl₂ solution. Cells were then grown in DMEM supplemented with 1% Fetal Bovine Serum, 1% non essential aminoacids and 1% Penicillin Streptomycin. Cells were fixed 24h, 48h or 72h post-transfection in 4% paraformaldehyde prepared in phosphate buffered saline (PBS) for 15 minutes and washed twice in phosphate buffered saline (PBS). Glass coverslips were incubated with a permeabilization solution containing PBS, 10% goat or donkey serum (Merck Millipore) and 0.1% triton for 30 min, one hour with primary antibodies diluted at appropriate concentration in the permeabilization solution. After three washes in PBS1X, coverslips were incubated for 1 hour with corresponding secondary fluorochrome conjugated antibody (Alexa Fluor) diluted at 1:1000. After 3 washes, the slides were incubated with Dapi and mounted with Fluoromount-G™ (Thermofisher Scientific) solution. Antibodies used for immunofluorescence include rabbit polyclonal anti-SOD1 (Merck Millipore #07-403-I) and sheep anti-SOD1 (Bioscience # K90077C). Fluorochrome-conjugated secondary antibodies were Alexa Fluor® 555 goat anti-rabbit and Alexa Fluor® 488 donkey anti-sheep (ThermoFisher). Images were collected with an apotome microscope (Zeiss AXIO Imager.M2) using a 63x (Plan-Apochromat, 63X/1.40 Oil DIC M27) objective with Hamamatsu Orca Flash LT+ camera (6.45µm pixel size, CMOS captor). Images were captured sequentially for each fluorescence channel under the following

illumination conditions: an LED-Module 385 nm for DAPI, an LED-Module 475 nm for Alexa Fluor 488, and an LED-Module 555 nm for Alexa Fluor 555. Each image of the plasmid constructs was taken with the same laser parameter exposure. A Z-stack composed of 1.5 μ m-layers were collected for each channel to obtain optical sectioning images through the cells. The resulting stacks were then processed and projected to generate composite images using ImageJ software.

Cryptic exon analysis

Available post-mortem fresh frozen slices of the spinal cord of patients were used in this analysis. Total RNAs were extracted from half transverse sections (including anterior and posterior horn) of spinal cords (1 mm of thickness) using a Polytron™ (Kinematica) homogenization in the TRIzol™ reagent (Invitrogen), followed by RNA purification and DNA removal with the on-column DNase I treatment (PureLink™ DNase Set, Invitrogen, Cat. No. 12185010) on the PureLink™ RNA Mini Kit columns (Invitrogen, #12183018A) according to the manufacturer's instructions. First-Strand cDNA synthesis was performed using SuperScript™ IV Reverse Transcriptase kit (Invitrogen, #18091050) on 100ng of RNA with a mix of oligo(dT) and random hexamers according to manufacturer's recommendations. RT-PCR for *STMN2* cryptic exon (*STMN2_{CE}*) and GAPDH were performed using primers previously reported (Supplemental Table S3) with Green Taq DNA polymerase (Fermentas), 40 cycles of amplification and 56°C as annealing temperature. Amplified fragments were analysed by electrophoresis on a 2% agarose with the GeneRuler 100 bp DNA Ladder (Thermofisher scientific). As *STMN2_{CE}* is normally not expressed, certain samples did not display any amplification of these aberrant transcripts. A sample was considered positive for cryptic exons when there was *STMN2_{CE}* product amplification in 3 independent PCR, each performed on an independent cDNA amplification.

Supplemental Table 1. Primary antibodies used in this study

CC1/CC2 are proprietary buffers from Ventana® : CC1 pH 8; CC2 pH 6.

Antigen	Poly/mono-clonal	Producer	Immunogen	Clone	Pretreatment	Dilution	Incubation time
TDP-43	Polyclonal (rabbit)	Proteintech®	N-terminal TDP-43		CC1® 8 min	1:2000	1h 20 min
Phosphorylated TDP-43	monoclonal (mouse)	Cosmo Bio Co®	pS409/410	11-9	CC1® 8 min	1:3000	1h
Poly-GA	Monoclonal (mouse)	EMD Millipore®	Polyethylene glycol-conjugated linear peptide containing poly-GA sequence	5E9	CC1® 36 min	1:100	36 min
Poly-GP	Polyclonal (rabbit)	Proteintech®	Peptide		CC2® 44 min	1:500	1h
Poly-GR	Polyclonal (rabbit)	Proteintech®	Peptide		CC2® 80 min	1:250	1h
SOD1	Polyclonal (rabbit)	Merk Millipore®	KLH-conjugated linear peptide corresponding to a C-terminal sequence of human Cu/Zn-SOD.	07-403-I	CC1® 36 min	1:1000	32 min
Misfolded SOD1	Monoclonal (mouse)	MediMabs®	Recombinant human SOD1 G93A APO	B8H10	Semi-manual, CC2® 44 min	1:500	Overnight +4°C
P62	Monoclonal (mouse)	BD Biosciences®	P62-Ick ligand	3/p62	CC2® 44 min	1:500	1h 36 min
Cystatin C	Polyclonal (rabbit)	Sigma®	precursor recombinant protein epitope signature tag (PrEST)		CC2® 44 min	1:50	32 min
Phosphorylated neurofilaments	Monoclonal (mouse)	Biolegend®	Phosphorylated neurofilaments H/M in axons	SMI310	CC1® 8 min	1:4000	1 h
Ubiquitin	Polyclonal (rabbit)	Dako®	Ubiquitin (cow erythrocytes)		CC1® 8 min	1:500	32 min
P-Tau	Monoclonal (mouse)	ThermoFisher®	Phospho-Tau (Ser202, Thr205)	AT8	CC1® 36 min	1:500	32 min
Beta-Amyloid	Monoclonal (mouse)	Abcam®	Amyloid betaA4	DE2B4	CC1®	1:15000	120 min
α-Synuclein	Monoclonal (mouse)	Fujifilm WAKO®	α-Synuclein	5G4	CC1®	1:3000	120 min
PolyQ	Monoclonal (mouse)	Millipore®	Polyglutamine repeats	STF1-1C2	Semi-manual, peroxidase blocking	1:3000	48 h
GFAP	monoclonal (mouse)	Agilent®	Glial Fibrillary Acidic Protein	6F2	CC1® 36 min	prediluted	28min
Iba1	Polyclonal (rabbit)	Fujifilm WAKO®	Synthetic peptide (Iba1 C-terminal sequence)		CC1® 64 min	1:500	32 min

Supplemental Table S2. Patient characteristics.

Cases	SOD1 variants	C9orf72 repeat expansion	sex	Age at death	Cause of Death	Site of onset	Disease duration (months)	MisSOD1 IHC	RT-PCR	References
SOD1^{p.T55I}	c.164C>T, p.Thr55Ile	G4C2 exp	M	60-64 y	ALS	LL	17 m	(+)	(+)	this study (*)
SOD1 ^{p.A5T}	c.13G>A, p.Ala5Thr	no	F	45-49Y	ALS	LL	17 m	(+)	(+)	
SOD1 ^{p.G94D}	c.281G>A, p.Gly94Asp	no	M	35-40y	ALS	LL	50m	(+)	(-)	42
SOD1 ^{p.insFLQ}	c.358-10T>G, p.Val119_Val120insPheLeuGln	no	M	55-59y	ALS	LL	11 m	(+)	(+)	42
SOD1^{p.Gly148Val}	c.443G>T, p.Gly148Val	G4C2 exp	F	65-69y	ALS	Bulbar	67 m	(-)	(-)	this study (*)
SALS1	no	no	F	65-69y	ALS	LL	160 m	(-)	(+)	
SALS2	no	no	F	60-64y	ALS	UL	83 m	(-)	(+)	
C9orf72-1	no	G4C2 exp	M	55-59y	ALS	UL	20 m	(+)	(+)	
C9orf72-2	no	G4C2 exp	M	60-64 y	ALS	LL	40 m	(-)	(+)	
C9orf72-3	no	G4C2 exp	M	50-54y	ALS	Bulbar	64 m	(+)	(-)	
Control-1	no	no	M	50-54 y	sudden death (pulmonary embolism)	N/A	N/A	(+)	(+)	
Control-2	no	no	F	90-94 y	stomach cancer	N/A	N/A	(+)	(+)	

Demographic, genetic and clinical information of post-mortem ALS cases analysed (+) or not (-) by misfolded SOD1 (misSOD1) immunohistochemistry (IHC) and/or RT-PCR and of ALS patient (* in bold) harboring both a *C9orf72* repeat expansion and a *SOD1* variant. M: male, F: female, y: years old, UL: upper limbs, LL: lower limbs, N/A: Not applicable.

Supplemental Table S3. Primers used for cryptic exon detection and SOD1 cDNA cloning in plasmids

Targets	Primers	Sequences (5'-3')	Amplicon Length	References
Stathmin-2 cryptic exon	Forward	GCCTCTTGCTCTTTCTCTAGCACG	179 pb	1, 41
	Reverse	TTTCTCTCGAAGGTCTTCTG		
GAPDH	Forward	GAAGGTGAAGGTCGGAGTC	226 pb	1, 33, 41
	Reverse	GAAGATGGTGATGGGATTTC		
SOD1 complete cDNA	Forward	GCGGAGGTCTGGCCTATAAAGTAGT	633 pb	42
	Reverse	TAACAGATGAGTTAAGGGGCCTCAG		
SOD1 cDNA for cloning p.T55I and p.FLQ	Forward	ATAGGATCCGCGCGCCATGGCGACGAAGGCCGTGT	494 pb	This study
	Reverse	TATTCTAGATCTCATTGGGCGATCCCAATTA		
SOD1 cDNA for cloning p.A5T	Forward	ATAGGATCCGCGCGCCATGGCGACGAAGGCCGTGT	494 pb	This study
	Reverse	TATTCTAGATCTCATTGGGCGATCCCAATTA		

Supplemental Table S4. Pathogenic impact of the variants mentioned in this study according to the ACMG classification.

Gene	Variant	Classification	Detailed ACMG Criteria [references]
SOD1	c.13G>A, p.Ala5Thr	Pathogenic	PS1 [32,35,43,47,50] PS3 [39, 45] PS4 PM1, PM2, PM5: p.A5D, p.A5S, p.A5V PP2, PP3, PP5
SOD1	c.59A>G, p.Asn20Ser	Uncertain	PS3 [39] PM1 PP2, PP3, PP5 BS1, BS2 BP4, BP6 [19, 51]
SOD1	c.164 C>T, p.Thr55Ile	Likely Pathogenic	PM1, PM2, PM5: p.T55R PP2, PP3
SOD1	c.281G>A, p.Gly94Asp	Pathogenic	PS1 [38,40,44,45,46] PS3 [39,42,45], PS4 PM1, PM2, PM5: p.G94A, p.G94C, p.G94R, p.G94S, p.G94V PP2, PP3, PP5
SOD1	c.358-10T>G, p.Val119Leu	Pathogenic	PS1 [34,42,48] PS3 [42] PM2, PM4: insertion FLQ PP1 [42], PP3, PP4 [42], PP5
SOD1	c.443G>T, p.Gly148Val	Likely Pathogenic	PM1, PM2, PM5: p.G148A, p.G148D PP2, PP3
DCTN1	c.1802G>C, p.Gly601Ala	Likely Pathogenic	PM1, PM2 PP3, PP5 [8]

Pathogenic criteria are divided into very strong (PVS1), strong (PS1–4), moderate (PM1–6), supporting (PP1–5); the benign criteria are divided into stand-alone (BA1 strong (BS1–4), supporting (BP1–7)).

Pathogenic Criteria:

PS1_Strong: Same amino acid change as a previously established pathogenic variant regardless of nucleotide change.

PS3_Strong: Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product.

PS4_Strong: The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls (case control studies)

PM1_Moderate: Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation

PM2_Moderate: Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium. *

PM4_Moderate: Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants

PM5_Moderate: Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before.

PP1_Supporting: Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease.

PP2_Supporting: Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease.

PP3_Supporting: Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc)

PP4_Supporting: Patient's phenotype or family history is highly specific for a disease with a single genetic etiology

PP5_Supporting: Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation

Benign Criteria

BS1_Strong: Allele frequency is greater than expected for disorder

BS2_Strong: Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age

BP4_Supporting: Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.)

BP6_Supporting: Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation

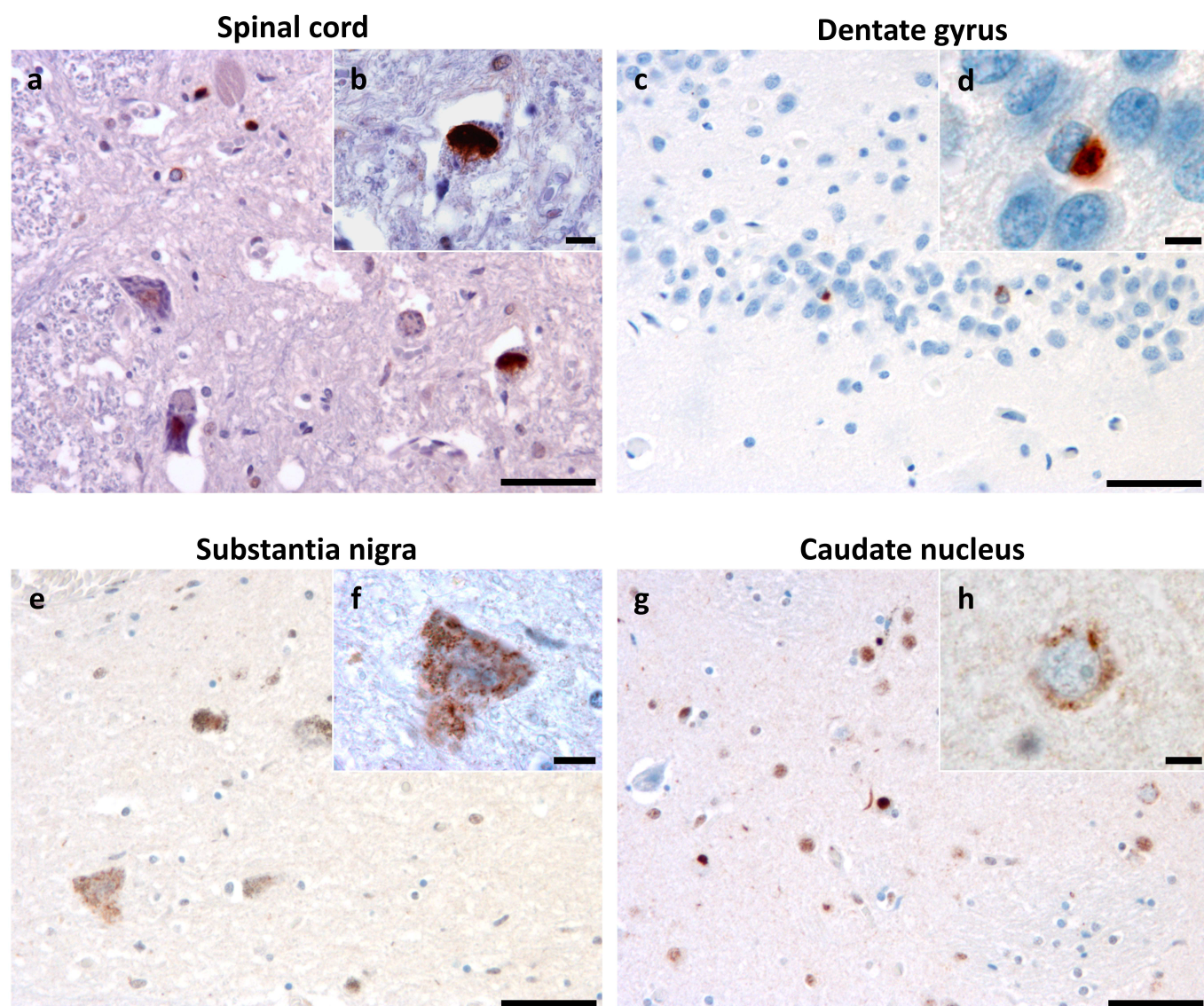
* frequency cut off was 0.00005 as recommended for ALS disease (36) determined in non finnish population (gnomAD V4)

Supplemental reference

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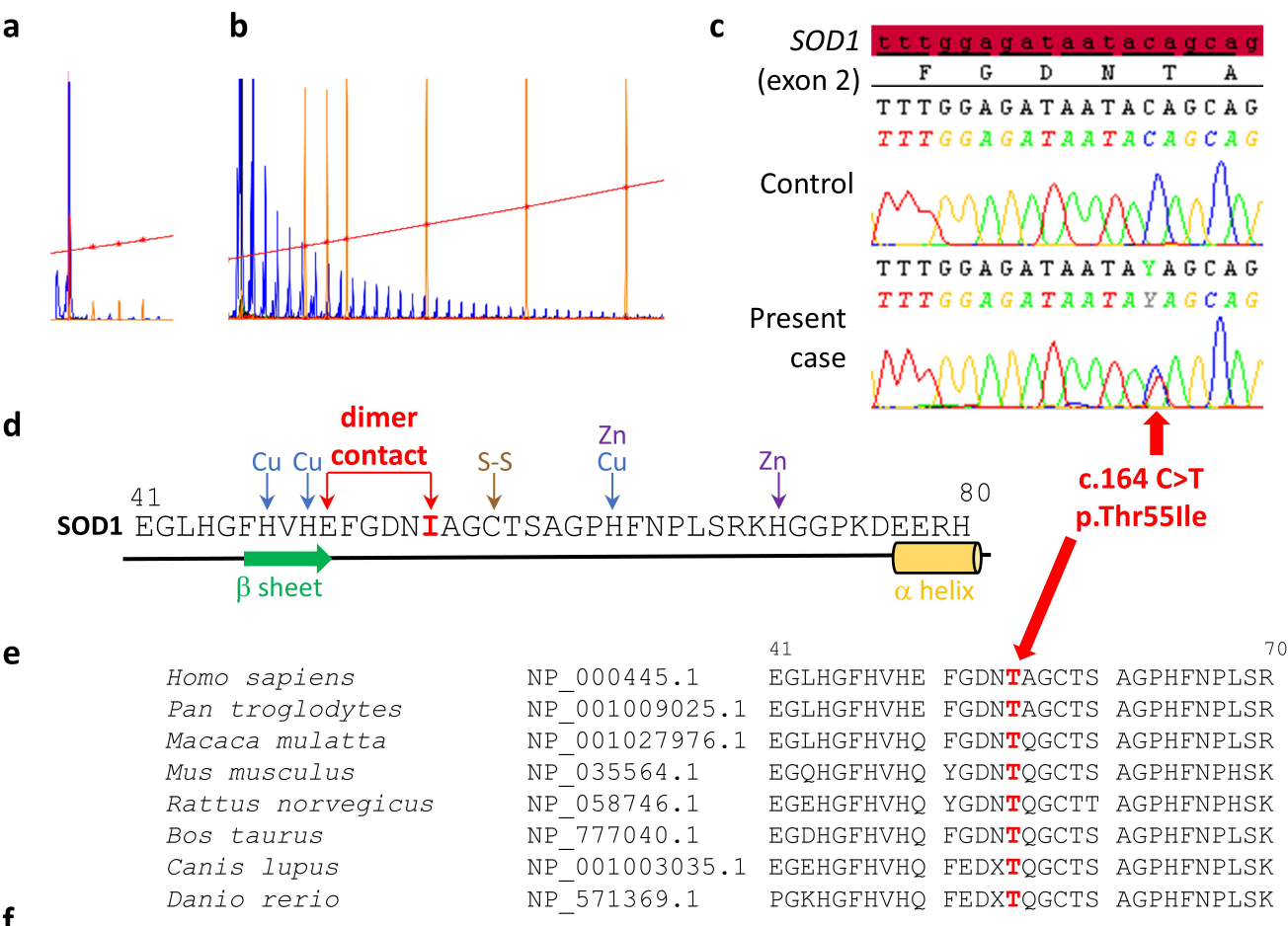


i

Motor cortex				Frontal cortex				Occipital cortex				Hippocampal DG				Hippocampal CA3/4			
NCI	NII	DN	GCI	NCI	NII	DN	GCI	NCI	NII	DN	GCI	NCI	NII	DN	GCI	NCI	NII	DN	GCI
2	0	2	1	3	0	2	2	0	0	1	0	2	0	0	0	0	0	0	0
Striatum				Substantia nigra				Hypoglossal nucleus				Spinal cord				Cerebellum			
NCI	NII	DN	GCI	NCI	NII	DN	GCI	NCI	NII	DN	GCI	NCI	NII	DN	GCI	NCI	NII	DN	GCI
3	0	2	2	2	0	1	1	2	0	1	2	3	0	1	3	0	0	0	1

Figure S1. Distribution of TDP-43 pathology in the p.Thr55Ile *SOD1/C9orf72* case.

Sections were stained with a-i) anti-phosphorylated TDP-43. a) Neuronal cytoplasmic inclusions (NCIs) and skein-like inclusions were found in motor neurons, and glial cytoplasmic inclusions (GCIs) were also frequently observed in the anterior horn of the spinal cord. B) NCI in motor neuron. C) A small number of NCIs were found in the dentate gyrus of the hippocampus. D) Round NCI. E) Some NCIs were detected in the substantia nigra. F) Diffuse NCI. G) Moderate NCIs were observed in the caudate nucleus. H) Diffuse NCI. i) Semiquantitative analysis of TDP-43 pathology in the p.Thr55Ile *SOD1/C9orf72* case. TDP-43-positive NCIs, neuronal intranuclear inclusions (NIIs), dystrophic neurites (DNs), and GCIs were semiquantitatively evaluated using the following grading system: 0, “absent”; 1, “scant” when a few lesions in the anatomical region; 2, “sparse” when lesions are present but not frequent in medium power field; 3, “moderate” when lesions are present in most medium power field; 4, “frequent” when many lesions are present in every medium power field. Scale bars = a,c,e,g 50 µm, b,f 10 µm, d,h 5 µm.



Tools	CADD score (v1.6)	Revel score (v2021)	AlphaMissense	SIFT (v6.2.0)	Polyphen2 (v2.2.8)	Mutation Taster (v2021)	PANTHER HMMs (v19.0)
<i>SOD1</i> p.Thr55Ile	26.3	0.948	Likely pathogenic	Deleterious	Probably damaging	Deleterious	Probably damaging
<i>DCTN1</i> p.Gly601Ala	27.4	0.792	Ambiguous	Deleterious	Probably damaging	Deleterious	Probably damaging

Figure S2. Genetic analyses revealed the co-occurrence of *C9orf72* expansions and predicted pathogenic missense variants in *SOD1* and *DCTN1*.

a,b) Peak scanner (v1.0) fluorescent profiles showed the patient carried 2 *C9orf72* alleles: the normal allele with 2 repeats (corresponding to the single peak amplified with primers flanking the repeat sequence, a), and, a second allele, with the expanded G₄C₂ hexanucleotide repeat, amplified after repeat-primed PCR (showing multiple fluorescent PCR products with typical 6 bp periodicity, b). The red line shows the GeneScan 500 ROX Size standard (Applied Biosystems). c) Parts of chromatograms after Sanger sequencing showing the position of the c.164 C>T variant (red arrow) in the exon 2 of *SOD1* compared to the nucleotide sequence of a control. d) Schematization of part of the mutated *SOD1* protein secondary structure (aa 41-80) compiled from the Jpred4 consensus prediction scores (<https://www.compbio.dundee.ac.uk/jpred/>) showing beta sheet (green arrow) and alpha helix (yellow tube) which were unchanged by the mutation, with positions of the dimer contact (red), copper (blue) and zinc (purple) binding sites, and disulfide bonds (brown) indicated. e) Part of aminoacid sequence alignment in various species showed the threonine at position 55 was highly conserved. f) Pathogenicity predictions of the *SOD1* p.T55I and the *DCTN1* p.G601A variants based on *in silico* analyses given by Alamut visual plus (v1.1) interface: Combined Annotation Dependent Depletion (CADD), Rare Exome Variant Ensemble Learner (REVEL), AlphaMissense (<https://alphamissense.hegelab.org>), Sorting Intolerant From Tolerant (SIFT), Polymorphism Phenotyping version 2 human diversity (Polyphen2-HDIV), MutationTaster, and Protein Analysis THrough Evolutionary Relationships (PANTHER).

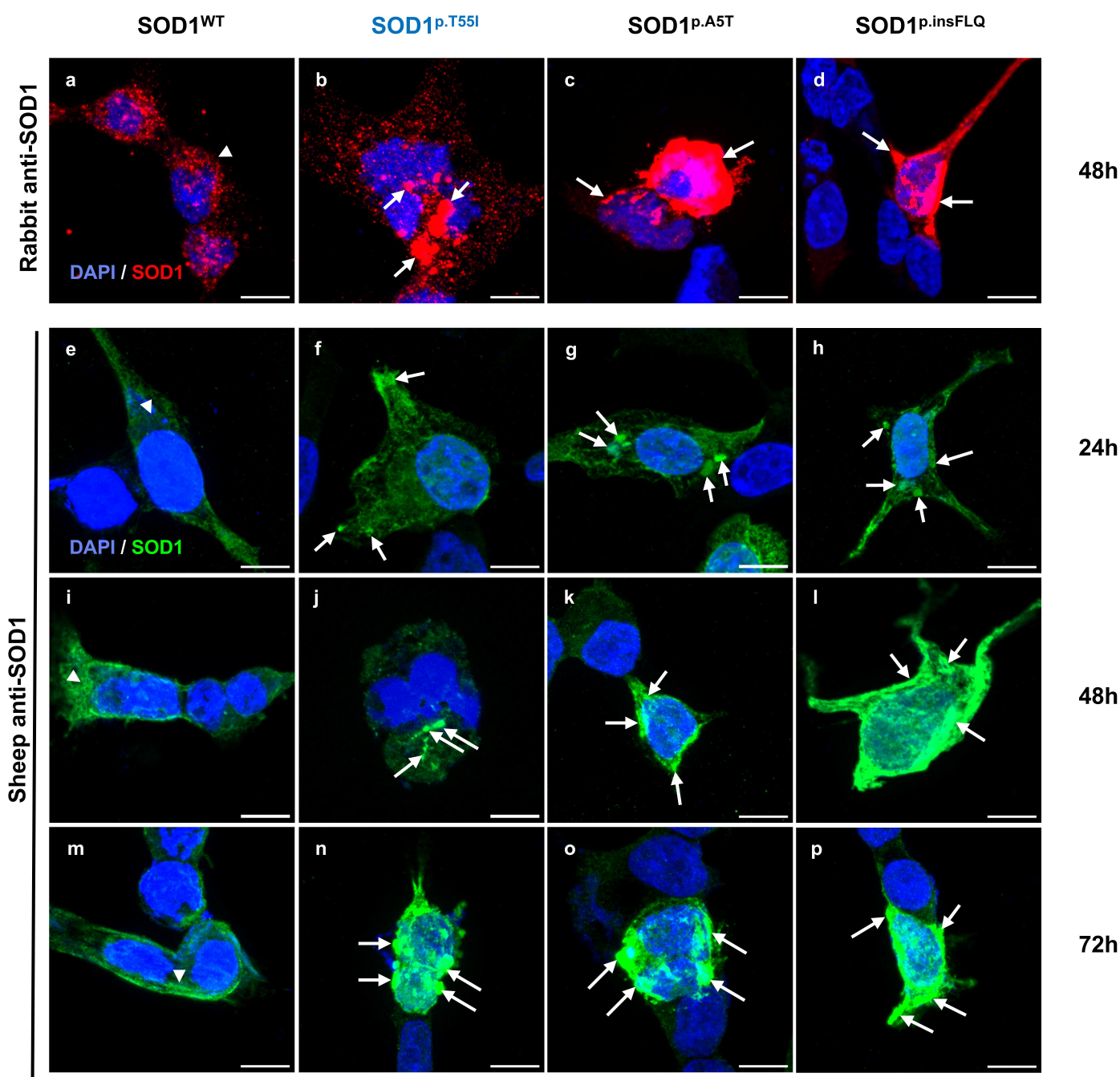


Figure S3. Representative images of HEK293 cells transfected with wild-type (WT) or mutant (p.T55I, p.insFLQ, p.A5T) SOD1 plasmid constructs. HEK293 cells were transfected with plasmids encoding wild-type (WT) SOD1 (a, e, i, m), SOD1 p.T55I (b, f, j, n) and p.A5T (c, g, k, o) missense variants, or p.Val119_Val120insPheLeuGln (p.insFLQ) SOD1 insertion variant (d, h, l, p). Cells were immunostained at various time points (24h to 72h after the transfections, as indicated on the right side of the images) using two different anti-SOD1 antibodies: rabbit anti-SOD1 used on human tissue (a-d, red) or sheep anti-SOD1 (e-p, green). Nuclei were counterstained with DAPI (blue). Arrowheads indicate diffuse SOD1 cytoplasmic staining, and arrows point to some SOD1 cytoplasmic inclusions in cells expressing mutant SOD1. Scale bar = 20 μ m.

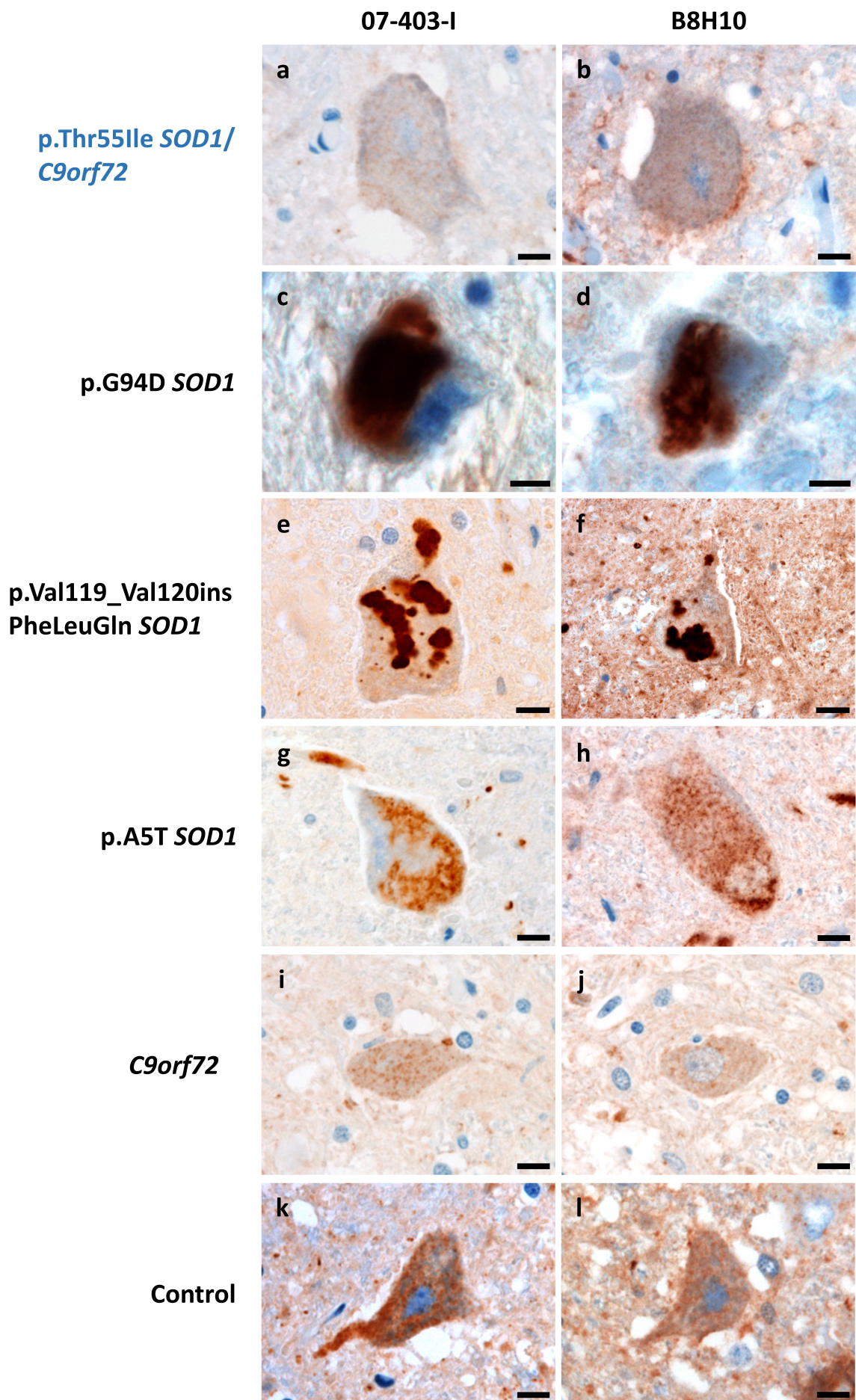


Fig.S4

Figure S4. SOD1 and misfolded SOD1 immunohistochemistry in the spinal cord in various ALS patients and control patients.

Sections were stained with a,c,e,g,i,k) anti-pan-SOD1 (07-403-I) and b,d,f,h,j,l) anti-misfolded SOD1 (B8H10). c,e,g) Pan-SOD1-positive neuronal cytoplasmic inclusions (NCIs) were observed in ALS cases with p.G94D, p.Val119_Val120insPheLeuGln, and p.A5T *SOD1* variant. d,f,h) These NCIs were also positive for the misfolded SOD1. a,i,k) Diffuse cytoplasmic SOD1 staining was slightly observed in the p.Thr55Ile *SOD1/C9orf72* case, ALS cases with *C9orf72* repeat expansion, and also in control cases. b,j,l) These small granular or diffuse cytoplasmic stainings were also labelled with anti-misfolded SOD1 antibody. a-l) anterior horn of the spinal cord. Scale bars = a,b,g-l 10 μ m, c,d 5 μ m, e,f 20 μ m.

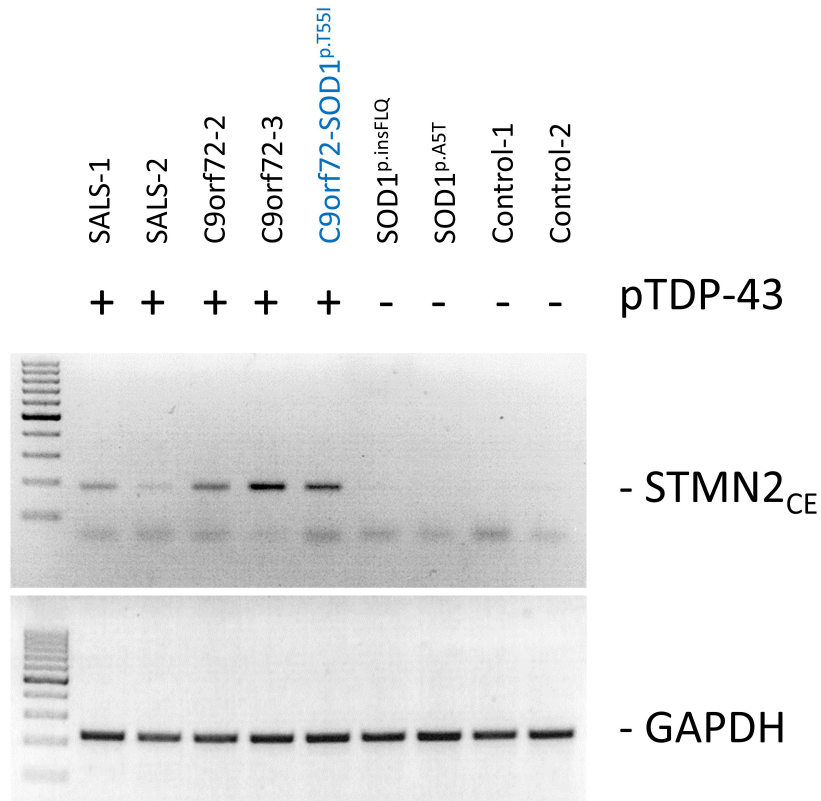


Figure S5. Analysis of STMN2 cryptic exons in post-mortem spinal cords.

RT-PCR performed on ALS spinal cords detected *STMN2* cryptic exon (STMN2_{CE}) in 2 sporadic (SALS-1 & -2) and 3 *C9orf72* patients including the *C9orf72* case carrying the p.Thr55Ile *SOD1* variant. No STMN2 cryptic exon was evidenced in controls and ALS cases with known pathogenic *SOD1* variants. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) amplification was used as standard control of the amplified cDNA. These results show *STMN2* is mis-processed in post-mortem spinal cords of cases associated with spinal phosphorylated TDP-43 (pTDP-43) pathology.