Transactive response DNA-binding protein-43 proteinopathy in oligodendrocytes revealed using an induced pluripotent stem cell model

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Oligodendrocytes are implicated in amyotrophic lateral sclerosis pathogenesis and display transactive response DNA-binding protein-43 (TDP-43) pathological inclusions. To investigate the cell autonomous consequences of TDP-43 mutations on human oligodendrocytes, we generated oligodendrocytes from patient-derived induced pluripotent stem cell lines harbouring mutations in the TARDBP gene, namely G298S and M337V. Through a combination of immunocytochemistry, electrophysiological assessment via whole-cell patch clamping, and three-dimensional cultures, no differences in oligodendrocyte differentiation, maturation or myelination were identified. Furthermore, expression analysis for monocarboxylate transporter 1 (a lactate transporter) coupled with a glycolytic stress test showed no deficit in lactate export. However, using confocal microscopy, we report TDP-43 mutation-dependent pathological mis-accumulation of TDP-43. Furthermore, using in vitro patch-clamp recordings, we identified functional Ca2+-permeable α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor dysregulation in oligodendrocytes. Together, these findings establish a platform for further interrogation of the role of oligodendrocytes and cellular autonomy in TDP-43 proteinopathy.

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Abbreviations: ALS = amyotrophic lateral sclerosis; AMPAR = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; DAPI = 4',6-diamidino-2-phenylindole; ECAR = extracellular acidification rate; FOV = fields of view; iPSC = induced pluripotent stem cell; MBP = myelin basic protein; NASPM = 1-naphthyl acetyl spermine; O4 = oligodendrocyte marker O4; OCR = oxygen consumption rate; OLIG2 = oligodendrocyte transcription factor 2; OPC = oligodendrocyte precursor cell; PBS = phosphate buffered saline; PDGF = platelet derived growth factor; TDP-43 = transactive response DNA-binding protein-43

Introduction

Neuronal and glial transactive response DNA binding protein-43 (TDP-43) inclusions are the pathological signature common to ~95% of ALS and ~50% of frontotemporal lobar degeneration cases. This includes disease due to a wide range of genetic mutations [TARDBP (encodes TDP-43 protein), PGRN, UBQLN2, SQSTM1, PFN1, ANG, VCP, MATR3, TUB4A and C9ORF72], which implicates shared disease mechanisms and a causative role for TDP-43 proteinopathy. A recent study using a transgenic TDP-43 mouse model demonstrated that whilst mutant TDP-43 in neurons was necessary for symptom onset, disease progression continued even after neuronal TDP-43 expression was reduced suggesting a strong pathogenic role for the surrounding glia. Until recently, the focus of non-cell autonomous mechanisms in ALS has been on the role of astrocytes and microglia.

Studies in post-mortem samples have demonstrated TDP-43 positive inclusions in oligodendrocytes, and a range of experimental and pathological studies have implicated oligodendrocyte lineage cells in the pathogenesis of ALS. These studies highlight both cell and non-cell autonomous processes. Furthermore, we, and others, have reported a subset of ALS patients who display exclusively oligodendroglial TDP-43 inclusions with no detectable neuronal TDP-43 proteinopathy. Animal studies have shown the critical importance of normal TDP-43 function in oligodendrocytes whereby selective deletion of TDP-43 from oligodendrocytes in mice causes motor impairments and changes to myelination. Along with the finding in a TDP-43Q331K ALS mouse model of upregulation in expression of myelination genes correlating with altered behaviour, these studies collectively implicate an important role for oligodendrocytes in ALS. Further, in demyelinating diseases, a key pathway driving oligodendrocyte injury is excitotoxicity. Excitotoxicity is a well-established pathway in ALS with an association between TDP-43 pathology and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) dysregulation. Our previous work has shown iPSC-derived motor neurons harbouring an expansion repeat mutation in the C9orf72 gene have a dysregulated AMPAR phenotype in favour of Ca$^{2+}$-permeable AMPARs leading to an increase in vulnerability to excitotoxicity. Further, there is regional and cellular heterogeneity of AMPAR dysregulation in ALS, with these data spanning...
iPSC models as well as human post-mortem tissue samples. Whether AMPAR dysregulation is present within oligodendrocytes exhibiting TDP-43 pathology remains to be determined. Any disruption to oligodendrocyte homeostasis could have detrimental effects on key functions such as myelin maintenance and metabolic capacity, both of which are critical for axonal health and function.

Here, we report the establishment and characterization of a human model to examine the cell autonomous consequences of TARDBP mutations in oligodendrocytes using two patient-derived iPSC lines each harbouring single point mutations in the TARDBP gene (G298S and M337V). Herein, we present a comprehensive assessment of development, morphology and function of oligodendrocyte harbouring mutations in the TARDBP gene, compared to appropriate controls. We show TDP-43 pathology in iPSC-derived oligodendrocytes, which is reversed in a CRISPR-Cas9 gene-edited control. Further, we identify a dysregulation in AMPARs in TDP-43G298S oligodendrocytes. Our data support a genotype-specific cell autonomous impairment in oligodendrocytes and establish a platform for further mechanistic studies.

**Materials and methods**

**Induced pluripotent stem cells generation, maintenance and gene editing**

Three patient-derived iPSC lines were used; six lines in total: one control line (Control), two clones from one TARDBP<sub>G298S</sub> patient line (ALS<sub>1</sub>G298S and ALS<sub>2</sub>G298S; male, 64 years old), two gene corrected lines from ALS<sub>2</sub>G298S (ALS<sub>2</sub>G298S-corr1 and ALS<sub>2</sub>G298S-corr2) and one clone from one TARDBP<sub>M337V</sub> patient line (ALS<sub>M337V</sub>; male, 59 years old). The derivation, characterization and validation of iPSC lines were performed as described. CRISPR/Cas9 gene editing in induced pluripotent stem cells

CRISPR/Cas9 mediated gene editing was performed on ALS<sub>2</sub>G298S iPSC clonal line. U6 promoter followed by gRNA (5’-GCTAGTTCGGGAACAATCA-3’) and gRNA scaffold was cloned into a pMTL23 vector through HindIII and XhoI restriction site cloning. ALS<sub>2</sub>iPSC line was dissociated using 1× Accutase (Sigma); 8 × 10<sup>5</sup> cells were nucleofected with 0.5 μg of pMTL23-gRNA, 1.5 μg of pSpCas9-2A-GFP (px458) plasmid and 100 pmol ssODN (180 bp repair template) using the Amaxa 4D nucleofector system (program CA137) following the manufacturer’s instructions. Following transfection, cells were plated on Matrigel (BD)-coated dishes in E8 medium supplemented with ROCK inhibitor (10 μM). Once cells reached confluency, they were again dissociated into a single cell suspension and plated at low density for clonal analysis. Each colony was screened for the accurate homologous recombination resulting in the replacement of the guanine at ORF position 892 with an adenine resulting in a gene correction from serine to glycine. The success rate of the CRISPR-Cas9 process was 0.7% and two colonies were expanded and maintained for use in experiments; one (ALS2<sub>G298S-corr1</sub>) had biallelic correction and the other (ALS2<sub>G298S-corr2</sub>) had monoallelic correction. By utilizing the two clones, we could guarantee that results were not due to CRISPR intervention and were a real result of the TARDBP<sup>G298S</sup> gene correction.

**Oligodendrocyte generation from induced pluripotent stem cells**

The patterning and derivation of oligodendrocytes from iPSC has been extensively described previously. Briefly, iPSC were lifted into suspension and underwent neuralization and caudalization. Neurospheres containing OLIG2<sup>+</sup> cells were treated with FGF2 (10 ng/mL), PDGF-AA (platelet-derived growth factor AA, 20 ng/mL, PeproTech), purmorphamine (1 μM, Sigma), SAG (1 μM, Calbiochem), IGF-1 (insulin-like growth factor-1, 10 ng/mL, PeproTech), heparin (5 μg/ml), T3 (triiodothyronine, 45 ng/mL, Sigma) to selectively pattern for oligodendrocytes. Terminal differentiation of oligodendrocytes was achieved by dissociation using the Papain Dissociation System (Worthington Biochemical) and plated on coated plates [Matrigel (BD Biosciences), Laminin (Sigma) and Fibronectin (Sigma)]; at this stage, 1 x ITS (Insulin-Transferrin-Selenium, Gibco) was added and all mitogens were removed except for T3 and IGF-1. A minimum of three independent differentiations were conducted per line for analyses.

**Electrophysiology**

To identify and visualize OPCs and oligodendrocytes within the cultures, OPCs and oligodendrocytes were stained live using PDGFRα or O4 antibodies and subsequently labelled with secondary Alexa 488 or Alexa 555 antibodies, respectively, prior to recording. The whole-cell patch configuration was used to record macroscopic currents as previously described. Current and voltage measurements were typically low-pass filtered online at 2 kHz, digitized at 10 kHz via a BNC-2090A (National Instruments) interface, and recorded to computer using the WinEDR V2 7.6 (J. Dempster, Department of Physiology and Pharmacology, University of Strathclyde, UK). Assessment of currents evoked by AMPAR were measured as detailed previously.
Quantitative RT-PCR

RNA was isolated from MACS-sorted O4-positive oligodendrocytes by using the RNeasy kit (Qiagen), following the manufacturer’s instructions. DNA contaminants were eliminated by using the Turbo DNA-Free kit (Invitrogen). cDNA was synthesized from 0.5 μg of total RNA with the DyNAmo cDNA Synthesis Kit (Thermo Scientific) in a 20 μL volume. Real-time quantitative polymerase chain reactions were performed in triplicate by using the DyNAmo Flash SYBR Green quantitative polymerase chain reactions kit (Thermo Scientific) and examined on a CFX96 System (Bio-Rad). Relative expression levels were calculated by the ΔΔCt method with GAPDH and 18S as the reference genes. Primer sequences used were:

| Primer       | Sequence                  |
|--------------|---------------------------|
| GAPDH_F      | 5'-GAG TCC ACT GGC GTC TTC AC |
| GAPDH_R      | 5'-ATG ACG AAC ATG GGG GCA T |
| 18S_F        | 5'-GTA ACC CGT TGA ACC CCA TT |
| 18S_R        | 5'-CCA TCC AAT CGG TAG TAG CG |
| TDP-43_F     | 5'-CGG CCT AGC GGG AAA AGT AAA AGA |
| TDP-43_R     | 5'-AGC ACC GTC CCA TCG TCT T |
| MCT1_F       | 5'-GAC CTT GGT GGA CCC CAG AG |
| MCT1_R       | 5'-AGC CGA CTC AAA AGT GGT GG |

Immunocytochemistry

All steps were performed at room temperature. Cells were fixed with 4% paraformaldehyde in PBS for 15 min, permeabilized with 0.3% Triton X-100 containing PBS and then blocked in 5% goat serum. This was followed by incubation with appropriate primary (TDP-43, Abnova #H0002343S-M01, 1:200; Olig2, Millipore #AB9610, 1:200; PDGFRα, Cell Signalling #5241S, 1:200; O4, R&D Systems #MAB1326, 1:500; MBP, Millipore #AB7349, 1:50) and secondary (Alexa Fluor 488, 1:1000; Alexa Fluor 647, 1:1000) antibodies. The nuclei were counterstained with DAPI (Sigma) and coverslips were mounted on slides with FluorSave (Merck).

Semi-quantitative densitometry analysis and Sholl analysis

Cells were imaged with an Axioscope (Zeiss) microscope or LSMZ10 Confocal microscope and processed with Axiovision v 4.8.1 (Zeiss). Semi-quantitative immunofluorescence analysis was performed using ImageJ64 (v1.45, NIH). Fields were selected based on uniform DAPI staining and imaged in three channels. For the analysis, images were first converted to grayscale and the DAPI and MBP channels were used to create regions of interest for quantification of cells harbouring granular TDP-43 cytoplasmic staining. Cell counts (Olig2, and PDGFα and MBP as a proportion of O4) were done manually using ImageJ64 (v1.45, NIH). Sholl analysis was performed on MBP+ cells using ImageJ with a starting radius 10 μm, step size 2 μm and sampled five times per radius.

Flow cytometry

Flow cytometry of oligodendrocytes to ascertain the population of O4-positive cells was performed on a FACScalibur (Becton Dickinson, San Jose, CA). Cells were gated with Accutase (Sigma) and stained with primary antibody O4 1:500 (R&D Systems; #MAB1326) and then secondary IgM (see above) 1:3000. Secondary and unstained controls were also performed. The cells were analysed by forward and side scatter for Alexa Fluor 488 fluorescence through a 530 ± 30 nm band-pass. Unstained cells were used to set the background fluorescence; a false positive rate of 0.5% was accepted. FACS data were collected and analysed using Cellquest and Flowjo software.

Glycolytic stress test

Following papain dissociation, cells were plated onto XF24 cell plates (Seahorse Bioscience, Billerica, MA) pre-coated with matrigel, laminin and fibronectin (as above). Optimization of reagents was performed and the protocols and algorithm program were used according to the XF24 analyser. Briefly, cells were transitioned from their oligodendrocyte specific media into the Agilent Seahorse Base Medium which has low buffering capacity and 0 mM glucose and maintained in ambient CO2 for 25 min. The plate was then inserted into the machine; baseline oxygen consumption rate and extracellular acidification rate were measured prior to the addition of glucose (10 mM), oligomycin (1.5 μM) and 2-deoxy-glucose (100 mM). Both oxygen consumption rate and extracellular acidification rate were measured three times following the injection of each drug.

Generation of induced pluripotent stem cells-derived organoids and assessment of myelination

We generated iPSC-derived organoids containing myelinating oligodendrocytes, termed ‘myelinoids’, using a previously published protocol. Briefly, the protocol mirrors the patterning protocol outlined above; after the selective patterning for oligodendrocytes, myelin induction was initiated by transferring individual spheres onto PTFE-coated cell culture membrane inserts (Millicell) in 6 well plates and cultured at 7.5% CO2. Spheres were maintained in this way for 12 weeks using a myelination media supplemented with 1 x ITS, 10 ng/mL IGF-1 and 45 ng/mL T3.

Analyses were conducted on whole-mounted and cryosectioned myelinoids. After 12 weeks of culturing in myelination media, myelinoids were fixed in 4% PFA for 2 h.
before washing and storing in PBS. For immunostaining, myelinoids underwent permeabilization in 0.3% triton-X-100 in PBS for 40 min before blocking in 10% normal goat serum in 0.25% triton-X-100 for 2 h. Antigen retrieval involved incubating myelinoids in citrate buffer (pH 6.0) at 95°C for 20 min followed by incubation in blocking solution again for 1 h. Primary antibodies (CNP, Atlas #AMAB91072, 1:2000; NF-H, Biolegend #822601, 1:10,000; CASPR, Abcam #AB34151, 1:1000) were diluted in blocking solution and incubated overnight at 4°C. Myelinoids were then washed in 0.1% Tween-20 before incubation in appropriate AlexaFluor secondary antibody (all used at 1:1000) for 2 h. Myelinoids were then incubated with DAPI for 10 min, washed in PBS and then mounted onto microscope slides (Thermo Scientific) with FluorSave (Calbiochem) and coverslipped (No, 1.5; Thermo Scientific).

Analyses were conducted as described previously. Briefly, for manual tracing of individual oligodendrocytes and nearest neighbour analysis, a Zeiss 710 confocal was used to take tiled z-stacks at 40× magnification. The Simple Neurite Tracer plugin was used in FIJI for manual tracing of CNP+ myelin sheath lengths per cell, and nearest neighbour analysis was performed by plotting a 100 μm radius circle around individual cells and counting the number of myelinating oligodendrocytes within that area.

Data availability
All data can be made available upon request to the corresponding authors.

Statistical analysis
Data are presented as mean ± S.E.M. Molecular, histological and metabolic outputs were analysed using a one-way ANOVA and Bonferroni’s multiple comparisons test or uncorrected Fisher’s least significant difference test for post hoc comparisons. Sholl data were compared using repeated-measures two-way ANOVA with Bonferroni’s multiple comparisons test for post hoc comparisons. Rectification indices of passive membrane currents were calculated from the relationship between the conductances of current data at −124 mV and +16 mV using the following equation: $I_{\text{rev}} / (I_{0} - I_{\text{rev}})$, where $I$ represents current amplitude, and $E_{\text{rev}}$ indicates the reversal potential of currents. Statistical comparison of the means was performed by Student’s t-test. * indicates $P < 0.05$; ** indicates $P < 0.01$; *** indicates $P < 0.001$. A number of biological and technical replicates are highlighted in figure legends whereby $N$ denotes number of independent differentiations and $n$ denotes number of cells or number of fields of view analysed.

Results
TARDBP mutant oligodendrocytes exhibit mutation-dependent TDP-43 cytoplasmic inclusions
We differentiated four iPSC lines (an unrelated control line [control], a patient line with an M337V point mutation in the TARDBP gene [ALS$^{\text{M337V}}$]) and two clones of a patient line with a G298S point mutation in the TARDBP gene [ALS$^{\text{G298S}}$, ALS$^{\text{G298S}}$] into oligodendrocytes using a method published previously. We also successfully used CRISPR-Cas9 to gene-edit the ALS$^{\text{G298S}}$ clone and then generated oligodendrocytes from two iPSC clones: one with mono-allelic and other with bi-allelic targeting (ALS$^{\text{G298S-corr1}}$: mono-allelic and ALS$^{\text{G298S-corr2}}$: bi-allelic; Fig. 1).

Using a method published previously (Fig. 2A), all lines efficiently generated oligodendrocytes (Fig. 2B) that expressed Olig2 (Control: 78.04 ± 0.71%; ALS$^{\text{M337V}}$: 71.91 ± 7.18; ALS$^{\text{G298S}}$: 59.37 ± 6.27; ALS$^{\text{G298S}}$: 60.69 ± 10.46; ALS$^{\text{G298S-corr1}}$: 72.04 ± 4.61; ALS$^{\text{G298S-corr2}}$: 71.32 ± 3.89).
Figure 2 Oligodendrocytes can be made from iPS lines harbouring a G298S mutation and an M337V mutation in the \( \text{TARDBP} \) gene. Following a previously published protocol, human-derived pluripotent stem cells were patterned towards the oligodendrocyte lineage; the final step of the process was differentiation from oligodendrocyte precursor cells to mature, post-mitotic oligodendrocytes (A). Lines were successfully patterned and markers of the oligodendrocyte lineage are shown in (B) for all lines. Scale bar = 50 \( \mu \text{m} \). There was no difference in percentage of Olig2 expressing cells (control \( N = 3 \), ALS\(^{M337V} \) \( N = 2 \), ALS1\(^{G298S} \) \( N = 2 \), ALS2\(^{G298S} \) \( N = 2 \), ALS2\(^{G298S-corr1} \) \( N = 2 \), ALS2\(^{G298S-corr2} \) \( N = 3 \); \( n > 500 \) cells counted per line) (C) or the percentage of O4 expressing cells (D) amongst lines (control \( N = 5 \), ALS\(^{M337V} \) \( N = 3 \), ALS1\(^{G298S} \) \( N = 4 \), ALS2\(^{G298S-corr1} \) \( N = 4 \), ALS2\(^{G298S-corr2} \) \( N = 4 \)). There was also no difference in co-expression of O4 and PDGFR\( \alpha \) (closed bars; control \( N = 3 \), ALS\(^{M337V} \) \( N = 2 \), ALS1\(^{G298S} \) \( N = 3 \), ALS2\(^{G298S} \) \( N = 3 \), ALS2\(^{G298S-corr1} \) \( N = 3 \), ALS2\(^{G298S-corr2} \) \( N = 3 \); \( n = 5-10 \) FOV counted per N) nor co-expression of O4 and MBP (open bars; control \( N = 3 \), ALS\(^{M337V} \) \( N = 4 \), ALS1\(^{G298S} \) \( N = 5 \), ALS2\(^{G298S} \) \( N = 3 \), ALS2\(^{G298S-corr1} \) \( N = 3 \), ALS2\(^{G298S-corr2} \) \( N = 2 \); \( n = 5-10 \) FOV counted per N) between lines 7 days after plate-down (E). Data are presented as average ± SEM.
We next examined for evidence of TDP-43 pathology in MBP-positive oligodendrocytes. Both ALS\textsuperscript{G298S} and ALS\textsuperscript{M337V} lines exhibited cytoplasmic mis-accumulation compared to control oligodendrocytes (Fig. 3A and B). Importantly, the cytoplasmic TDP43 inclusions observed in TARDBP\textsuperscript{G298S} oligodendrocytes were reverted back to the control levels in the gene-corrected clones (Fig. 3B). Despite the increase in cytoplasmic TDP-43 inclusions, there was no compensatory increase in TDP-43 mRNA expression (Fig. 3C).

**Cell-autonomous consequences of TDP-43 mutation on oligodendrocyte morphology, electrical activity, metabolic function and myelinating capacity**

Glutamate receptor dysregulation is a major hypothesis driving ALS pathogenesis. This study is the first to examine AMPAR dysregulation in oligodendrocytes harbouring mutations in the TARDBP gene (Fig. 4).

We assessed the proportion of Ca\textsuperscript{2+}-permeable AMPARs in OPCs (PDGFR\textsuperscript{α+} cells) and mature oligodendrocytes (O4\textsuperscript{+}/PDGFR\textsuperscript{α−} cells) by measuring the block of AMPA-mediated currents by Ca\textsuperscript{2+}-permeable AMPAR channel blocker 1-naphthyl acetyl spermine (Fig. 4A).\textsuperscript{26} It was found that upon differentiation of OPC to mature oligodendrocytes, there was the normal transition from Ca\textsuperscript{2+}-permeable to Ca\textsuperscript{2+}-impermeable AMPAR in control (black bars) and ALS\textsuperscript{M337V} (purple bars). In contrast, the ALS\textsuperscript{G298S} oligodendrocytes maintain functional expression of Ca\textsuperscript{2+}-permeable AMPARs (Fig. 4A and B; orange bars). Importantly, this phenotype was rescued in ALS\textsuperscript{G298S-corr} oligodendrocytes with the level of 1-naphthyl acetyl spermine block mirroring the ALS\textsuperscript{G298S-corr1} inogenic line (representative image of an patient line and its CRISPR-Cas9 gene-edited control line ALS\textsuperscript{G298S-corr1}. We observed widespread myelin formation in our 3D organoid model derived from both ALS\textsuperscript{G298S} and ALS\textsuperscript{G298S-corr1} isogenic line (representative image of an ALS\textsuperscript{G298S-corr1} myelinating organoid in Supplementary Fig. 2A and higher magnification images of myelinating oligodendrocytes from ALS\textsuperscript{G298S} and ALS\textsuperscript{G298S-corr1} in Supplementary Fig. 2B) with intact paranode formation as shown by the presence of CASPR at the distal ends of myelin sheaths (Supplementary Fig. 2C). We also noted no statistical difference in oligodendrocyte density between the two

harbouring a TARDBP\textsuperscript{G298S} mutation. Surprisingly, ALS\textsuperscript{M337V} oligodendrocytes did not show an equivalent dysregulation (OPC data 5.96 ± 0.16 pS, OL data 3.26 ± 0.19 pS; Fig. 4D). Therefore, the dysregulation in AMPAR was specific to the G298S genotype, and its persistence through to 3-week-old oligodendrocytes suggests it was not due to delayed maturation of AMPAR composition in oligodendrocytes.

To further verify that the differences in AMPAR were not due to impairments in maturation in TARDBP\textsuperscript{G298S} oligodendrocytes, we next assessed the membrane properties of the cells.\textsuperscript{22} We found no correctable difference in cell capacitance (Supplementary Fig. 1A and B), nor membrane resistance (Supplementary Fig. 1C) or rectification of membrane currents (Supplementary Fig. 1D), indicating that the AMPAR dysregulation was not a result of impaired maturation of oligodendrocytes. Further, we also demonstrate no difference in oligodendrocyte morphology with no correctable difference in cell size or number of processes measured using Sholl analysis (Supplementary Fig. 1E) indicating that the AMPAR dysregulation was also not a result of changes to cell morphology.

Oligodendrocytes are increasingly recognized to provide metabolic support to neurons with some evidence for dysregulation in ALS.\textsuperscript{14,28–30} To determine if there was a mutant-dependent effect on metabolic function, we first measured expression of the key lactate transporter MCT1 and found no difference between control and mutant lines (Fig. 3A). Next, we used the Agilent Seahorse XF Analyzer to assess changes in extracellular acidification rate representative of basal glycolysis, maximum glycolytic capacity and glycolytic reserve of oligodendrocytes by addition of glucose, oligomycin and 2-deoxy-glucose, respectively. No difference between any of the lines at any of the assay timepoints was observed (Fig. 3B). Quantification of the gradient shift in extracellular acidification rate after addition of glucose (representing basal glycolysis) was not different between genotypes (Fig. 3C).

To evaluate whether mutations in the TARDBP gene impaired an oligodendrocyte’s capacity to myelinate, we used a recently reported organoid system\textsuperscript{27} that reproducibly yields widespread and compact myelin with nodal organization. Given the more severe phenotype in the G298S oligodendrocytes, we chose to focus on the ALS\textsuperscript{G298S} patient line and its CRISPR-Cas9 gene-edited control line ALS\textsuperscript{G298S-corr1}. We observed widespread myelination in our 3D organoid model derived from both ALS\textsuperscript{G298S} and ALS\textsuperscript{G298S-corr1} isogenic line (representative image of an ALS\textsuperscript{G298S-corr1} myelinating organoid in Supplementary Fig. 2A and higher magnification images of myelinating oligodendrocytes from ALS\textsuperscript{G298S} and ALS\textsuperscript{G298S-corr1} in Supplementary Fig. 2B) with intact paranode formation as shown by the presence of CASPR at the distal ends of myelin sheaths (Supplementary Fig. 2C). We also noted no statistical difference in oligodendrocyte density between the two
cell lines (Supplementary Fig. 2D). Next, by manually tracing sheaths of individual oligodendrocytes, we found no difference in the number or mean length of internodes produced by each oligodendrocyte (Supplementary Fig. 2D and E). The frequency distribution of internode lengths in G298S oligodendrocytes was also unaffected (Supplementary Fig. 2F). Taken together, these results demonstrate that the TARDBP mutation has no effect on myelin formation.

**Discussion**

We report the demonstration of TDP-43 cytoplasmic mislocalization and selective genotype-dependent evidence of AMPAR dysregulation in ALS patient iPSC-derived oligodendrocytes. These findings are consistent with both previous human autopsy studies characterizing TDP-43 in sporadic ALS cases and confirm recapitulation of TDP-43 proteinopathy in both motor neurons and astrocytes derived from TDP-43 mutant iPSC lines and astrocyte cultures.

Despite oligodendrocytes exhibiting TDP-43 protein mislocalization, there was no demonstrable deficit on maturation, morphology, metabolic function or myelination. We did find an effect on AMPAR properties implying a potential contributing role of oligodendrocytes to the excitotoxicity phenotype characteristic of ALS. The literature implicating oligodendrocytes in ALS is brief, but growing, and spans human post-mortem tissue, mouse models, and is only beginning to be modelled using iPSC. Collectively, it suggests a cell autonomous and non-cell autonomous deficit in oligodendrocytes in ALS. Our electrophysiological data suggesting oligodendrocytes harbouring a G298S point mutation in the TARDBP gene,
iPSC-derived oligodendrocytes and ALS

Figure 4 ALS<sup>G298S</sup> oligodendrocytes, but not ALS<sup>M337V</sup> oligodendrocytes exhibit Ca<sup>2+</sup>-permeable AMPARs receptors but this is not due to maturation deficits. (A) Example recording of NASPM block of steady-state currents evoked by AMPA in week 3 O4<sup>+</sup>-oligodendrocytes derived from control (black), TDP-43<sup>G298S</sup> (orange) and TDP-43<sup>G298S-corr</sup> (blue). Scale bar (control) of 25 pA, 5s; scale bar (TDP-43G298S) of 50 pA, 10s, and scale bar (TDP-43G298S-corr) of 20 pA, 10s. (B) Mean (±SEM) percentage NASPM block of AMPA currents in PDGFR<sup>α</sup> positive oligodendrocyte precursor cells (examined at week 1; solid bars; control N = 4 n = 12, ALS<sup>M337V</sup> N = 1 n = 6, but not an M337V mutation, retain Ca<sup>2+</sup>-permeable AMPAR is of interest. AMPAR dysregulation in genetic and sporadic ALS has previously been demonstrated in neurons<sup>21,23,34</sup> but our data are the first to implicate oligodendrocytes, albeit with genotype specificity. Indeed, similar to our data, the G298S genotype has previously been found to cause demyelination but also preserve axonal integrity.37 Investigating this phenomenon further in an ALS context by utilizing co-culture systems and in vivo models is imperative.

The absence of deficits in glycolytic function is in contrast to findings from Ferraiuolo and colleagues<sup>13</sup> who...
found that iPSC-derived oligodendrocytes with a TDP-43 mutation produce less lactate than comparative controls. This difference could be attributed to the difference in the challenge to the cells; Ferraiuolo and colleagues measured basal lactate concentrations whereas the Seahorse assay is a glycolytic stress test so has measured the cells’ ability to undergo glycolysis whilst under stress. Further, our patterning protocol produces a cell population that contains a proportion of astrocytes. Both studies focus on pre-myelinating oligodendrocytes, so more sophisticated and/or longer-term assays of oligodendrocyte behaviour that allow assessment of myelin generation and maintenance are required to reveal the subsequent deleterious effects of a TDP-43 mutation in oligodendrocyte lineage cells on both myelination as well as metabolic capacity. This is particularly important given that despite we, and others, showing no change to MCT1 expression in iPSC-derived oligodendrocytes harboring TARDBP mutations, in end-stage mouse and human disease MCT1 protein levels were found to be decreased. Myelinating organoid experiments revealed no effect of a TARDBP mutation on myelination and the generation of myelin internodes. This is consistent with a recent rodent study that found no effect of selective TDP-43 deletion in oligodendrocytes on paranodal assembly. Further studies are needed to ascertain myelination status and the influence of TARDBP mutation over time to model prolonged disease.

In summary, we report recapitulation of TDP-43 pathology in oligodendrocytes that is TDP-43 mutation dependent establishing a platform for further studies in the arena of both disease modelling and drug discovery. Further, we identified a genotype-specific AMPAR phenotype highlighting a cell autonomous impairment in oligodendrocytes that emphasizes the importance of considering oligodendrocytes as a key player in ALS pathogenesis.

Supplementary material
Supplementary material is available at Brain Communications online.

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Competing interests
S.C. is a co-founder of Pheno Therapeutics. All other authors report no competing interests.

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