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# Motor Neuron Disease 1

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**WFN15-0257**
**Motor Neuron Disease 1**
**Modelling amyotrophic lateral sclerosis (ALS) using mutant and CAS9/CRISPR-corrected motor neurons from patients with C9ORF72 mutations reveals disease-specific cellular phenotypes**

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**Background**: The C9orf72 hexanucleotide expansion is the commonest genetic cause of ALS and Frontotemporal Dementia (FTD). In addition to cytoplasmic aggregation of phospho-TDP-43, pathological features include RNA foci and aggregations of dipeptide protein. The relative contribution of these pathologies to the disease remains unresolved.
**Objective**: To use human motor neurons from patients with ALS, and correction with gene editing, to resolve the key pathological features of ALS.
**Methods**: Induced pluripotent stem cell (iPSC) lines were generated from four ALS patients carrying the C9ORF72 repeat expansion. One line was corrected by genome editing to serve as an isogenic control. Cells were characterized functionally and pathologically.
**Results**: ALS/FTD iPSC line OXC9-02-02 was successfully used to target the expanded G4C2 repeat using CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9-mediated homologous recombination, in the presence of plasmid DNA donor template containing a positive selection cassette. In *C9orf72* iPSC-derived motor neurons, dysfunction in $Ca^{2+}$ homeostasis and endoplasmic reticulum tER) stress correlated with decreased cellular survival and reduced levels of the anti-apoptotic protein Bcl-2. Furthermore, the *C9orf72* motor neurons showed evidence of abnormal protein aggregation and stress granule formation in the absence of external stress. These phenotypes were corrected by excision of the mutation by gene editing.
**Conclusions**: We have demonstrated that genome editing can be used to validate an ALS/FTD model system. The identification of a novel pathogenic link between *C9orf72* mutations, dysregulation of calcium signalling and altered proteostasis demonstrates the value of iPSC-derived motor neurons as a cellular model for the investigation of neurodegeneration.

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## 123
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**Motor Neuron Disease 1**
**Expression and subcellular localization of FUS protein in fibroblasts of preclinical FUS P525L mutation carriers and patients with sporadic ALS**

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**Background**. Symptom onset in Amyotrophic Lateral Sclerosis (ALS) occurs when over 70% of motor neurons are lost. This suggests an extended preclinical phase, in which no cognitive, electrophysiological or neuroimaging changes are detectable (Eisen A et al., 2014). Furthermore, the availability of genetic testing allows identification of mutation carriers, which might help to understand the molecular changes preceding the clinical onset.
**Objective**. To study the expression of FUS protein in skin fibroblasts from preclinical carriers of FUS P525L mutation, a healthy control and patients with sporadic ALS (sALS).
**Patients and methods**. Skin fibroblasts from two healthy sisters carrying a FUS P525L mutation and two patients with sALS were cultured. As a control, fibroblasts were taken from a healthy man with no known ALS-related mutations. Western blot and immunocytochemistry were performed to study the expression and subcellular localization of FUS protein in fibroblasts from mutation carriers, control and sALS patients.
**Results**. In sALS, FUS protein showed an almost exclusive nuclear localization, where it also forms aggregates. FUS expression was mostly nuclear in control fibroblasts, with a relatively weak cytoplasmic expression. In the two FUS P525L mutation carriers, a high proportion of cells showed a prominent protein localization in both nucleus and cytoplasm, or in the cytoplasm alone.
**Conclusions**. FUS protein is differentially localized in fibroblasts from P525L carriers with respect to healthy control and sALS. In the mutation carriers, FUS is often mislocalized in the cytoplasm. This represents the first evidence of specific molecular changes occurring in preclinical ALS.

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**WFN15-0948**
**Motor Neuron Disease 1**
**Reaching and grasping a glass of water by locked-in ALS patients through a BCI-controlled humanoid robot**

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**Background**. Amyotrophic lateral sclerosis (ALS) leads to a complete limb paralysis, dysphagia and anarthria. A brain-computer interface (BCI) technology may aid ALS patients in communication and motor control.
**Objective**. To set up a BCI system for wireless control, by ALS patients, of a humanoid robot, with the aim to reach and grasp a glass of water.
**Patients and methods**. Four non-demented ALS patients were recruited. Controls were four healthy subjects, matched for demographic variables. A BCI command interpreter was used to control a humanoid robot. The task was to instruct the robot to move towards a glass of water, reach and grasp it (first item) and then bring the glass to the subject (second item). The protocol consisted of a calibration session, an online session, in which the two items are sequentially selected, and a robot session where the two items translate into high level commands. The minimal accuracy of each response and the number of errors each session were evaluated and analysed.
**Results**. All ALS patients completed the task (5 trials, 95.5% success). Controls performed comparably with a 100% success over the 5 trials. The minimum accuracy leading to a correct item selection for the robot movement was slightly better for ALS patients (ALS 60% vs controls 53%, $p = 0.49$).
**Conclusions**. ALS patients can successfully control a humanoid robot through a BCI system. This bears the potential to virtually restore the autonomous motion of an ALS patient, enabling him to extend his presence beyond the boundaries of his bed.

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## 125
## WFN15-1342
## Motor Neuron Disease 1
## Fibroblasts from patients with amyotrophic lateral sclerosis (ALS) associated with mutations in tardbp gene as model of TDP-43 proteinopathy

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**Rationale & hypothesis:** ALS is a devastating progressive neurodegenerative condition, which results in death. TARDBP encoded protein, TDP-43, has been implicated in both the sporadic and familial ALS cases. Animal models of TDP-43 have been inconclusive and the role of TDP-43 in ALS remains an enigma to date. Fibroblasts obtained from the patients carrying mutations in TARDBP gene provide a vital tool in investigation of TDP-43 due to physiological levels of TDP-43.
**Methodology:** Immunocytochemistry was performed on three lines of control and three different TDP-43 mutant fibroblast lines (M337V, G287V, A321V) and confocal microscopy was performed to identify general TDP-43, phosphorylated TDP-43 and anti p62 (to identify ubiquitin) localisations. Fibroblasts were also subjected to 0.5 mM arsenite and the stress response was assessed using markers of stress granules such as TIAR and HUR. Recovery after stress was also assessed.
**Findings/conclusion:** In keeping with findings in ALS postmortem material, relative clearing of nuclear TDP-43 was noted in mutant TDP-43 fibroblasts ($p < 0.001$). TDP-43 fibroblasts also showed accumulation of p62 positive aggregates ($p < 0.0003$), and phosphorylated TDP-43 accumulation ($p < 0.001$) compared to controls, suggesting that mutant TDP-43 fibroblasts share some characteristics of the surviving motor neurons from both sALS and fALS. Following exogenic stress endogenous TDP-43 localised to HUR positive stress granules ($p < 0.01$), formation of stress granules and their recovery were significantly impaired in mTDP-43 cases ($p < 0.01$) suggesting that dysfunction of TDP-43 dysregulates handling of exogenic stress. We suggest that this may contribute to premature degeneration of motor neurons expressing mutant TDP-43 in ALS patients. Fibroblasts also form a robust and an economical platform to study TDP-43 related neurodegeneration.

I have obtained patient and Institutional Review Board (IRB) approval and local ethics committee approval.

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## 126
## WFN15-1481
## Motor Neuron Disease 1
## C9ORF72 repeated expansion in patients with familial amyotrophic lateral sclerosis from a Brazilian research center. A preliminary report

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**Background**: The expanded GGGGCC hexanucleotide repeat in the non-coding region of the chromosome 9 open reading frame 72 (*C9ORF72*) gene is the most common genetic abnormality in familial amyotrophic lateral sclerosis (FALS).
**Objective**: To determine the *C9ORF72* hexanucleotide repeat expansion in FALS patients from ALS Unit of São Paulo, Brazil.
**Patients and methods**: Patients with FALS from the ALS Unit of Clinics Hospital, University of São Paulo Medical School, Brazil have been evaluated for the presence of an expanded (GGGGCC) in *C9ORF72*. A repeat-primed-PCR reaction was applied to provide a qualitative assessment of the expansions. PCR products were analyzed on an ABI3730 and visualized using GeneMapper-software. A cutoff of >30 repeats combined with a typical sawtooth pattern was considered pathologic.
**Results**: Preliminaries results from 15 FALS patients (mean age of onset $51.40 \pm 3.02$ years) are shown. The repeat expansion was present in 5 FALS cases (33.3%) and 10 FALS did not present the pathologic expansion. One patient with *C9ORF72* expansion presented a bulbar-onset and developed later a frontotemporal degeneration. Patients without *C9ORF72* expansion had a spinal-onset disease. FALS patients with *C9ORF72* expansion developed a later onset symptoms ($54.80 \pm 4.16$ years) when compared to FALS without expansion ($49.7 \pm 4.07$ years). A shorter lifespan was screen in *C9ORF72* expansion carriers ($5.0 \pm 1.22$ years) than *C9ORF72* negative ($9.10 \pm 2.42$ years).
**Conclusion**: A high frequency of *C9ORF7* expansion was detected in this partial report of a small FASL sample of São Paulo ALS Unit. Supported by FAPESP and CNPq, Brazil.

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## 127
## WFN15-0089
## Motor Neuron Disease 1
## Epidemiologycal and clinical features of amyotrophic lateral sclerosis in Uzbekistan

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by multifactorial etiology, affections of central and