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Motor Neuron Disease 1

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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

R. Mutihac[a], N. Ababneh[a], J. Scaber[a], R. Wade-Martins[b], S. Cowley[c], K. Talbot[a,*]. [a]*Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, United Kingdom;* [b]*Department of Physiology Anatomy and Genetics, University of Oxford, Oxford, United Kingdom;* [c]*James Martin Stem Cell Facility, University of Oxford, Oxford, United Kingdom*

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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Expression and subcellular localization of FUS protein in fibroblasts of preclinical FUS P525L mutation carriers and patients with sporadic ALS

V. La Bella[a,*], M. Lo Bello[a], F. Di Fini[a], F.L. Conforti[b], R. Spataro[a]. [a]*BioNeC, ALS Clinical Research Center, Palermo, Italy;* [b]*National Research Council, Institute of Neurological Sciences, Mangone, Italy*

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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Reaching and grasping a glass of water by locked-in ALS patients through a BCI-controlled humanoid robot

R. Spataro[a,*], R. Sorbello[b], S. Tramonte[b], G. Tumminello[c], M. Giardina[b], A. Chella[b], V. La Bella[c]. [a]*ALS Clinical Research Center, University of*

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