Characterisation of a novel genetic variant of amyotrophic lateral sclerosis within a new disease pathway

Tobias Moll, Dr Johnathan Cooper-Knock, Dr Alexander Beer, Dr Henry Robbins, Dr Adrian Higginbottom, Dr Guillaume Hautbergue, Dr Lydia Castelli, Dr Tennore Ramesh, Dr Janine Kirby, Prof Dame Pamela Shaw

Department of Neuroscience, University of Sheffield

INTRODUCTION

Current understanding of motor neuron degeneration in amyotrophic lateral sclerosis (ALS) is based primarily on the study of genetic subtypes. Discovering new genetic causes of disease offers a powerful platform for building disease models and identifying therapeutic targets, particularly when the new gene occurs in a distinct functional pathway.

AIMS & OBJECTIVES

To identify and characterise a novel genetic variant of ALS. To develop cell and animal models to elucidate pathological mechanisms linking the novel genetic variant to motor neuron loss.

METHODOLOGY

Whole exome sequencing of DNA donated by two related individuals with autosomal dominant ALS was followed by targeted sequencing of candidates in a cohort of 103 familial and young sporadic ALS cases. Once a candidate gene was identified: We used immunocytochemistry to study the intracellular localisation of the wild-type protein. The functional impact of the mutations were tested in neuronal (N2A) and non-neuronal (HEK-293) cell lines via MTT and lactate dehydrogenase (LDH) assays. Knockdown of the gene in zebrafish embryos was performed using morpholino oligonucleotides (MO).

FINDINGS

Only one genetic variant was present in both family members with autosomal dominant ALS, absent from controls (n=220 local, n=60,000 in ExAC), and found in additional ALS cases (n=4) (Fig. 1). In advance of the first publication, the gene shall be referred to as 'X'. No patients with a mutation in gene X had a coexisting mutation in a known ALS gene, and disease was within the clinical spectrum of sporadic ALS. 5 patients with p.R92C mutations suffered more aggressive disease with mean survival of 13 months, but the single patient with a p.G78W mutation lived >5 years. Immunocytochemistry showed possible localisation of protein X to the Golgi network in neuronal and non-neuronal cells (Fig. 2). Mutant forms are more cytotoxic than the wild-type, and reduce cellular metabolism (Figs. 3, 4). The p.R92C mutation was more toxic than p.G78W in all assays which is in-line with observed clinical severity. Knockdown of gene X reduced motor activity in zebrafish embryos at 5 days post fertilisation (Fig. 5).

Fig. 1: Pedigree for the original family in which mutations were discovered. Exome sequencing was performed in two related individuals with ALS (*). Subsequent Sanger sequencing (of all members denoted by red shapes) confirmed the mutations in the ALS patients (shaded grey) and showed that mutations were absent from unaffected individuals.

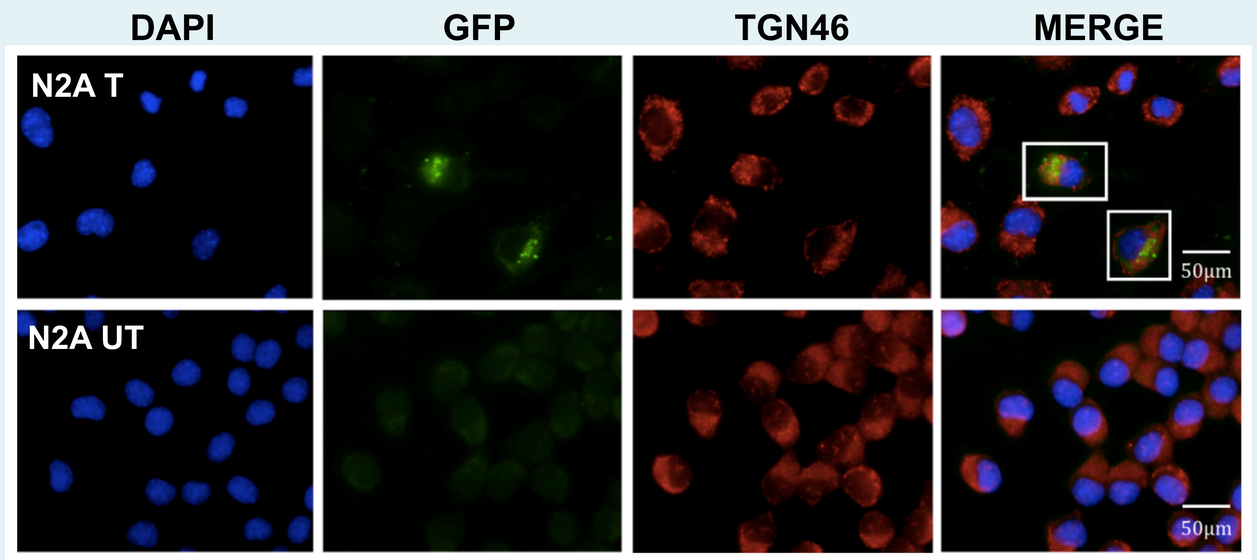

Fig. 2. Protein X may be localised to the Golgi network in N2A cells. Golgi network labelled using anti-TGN46 (red); protein X-GFP labelled with anti-JL8 (green); nuclear counterstain in blue; scale bar is 50μm.

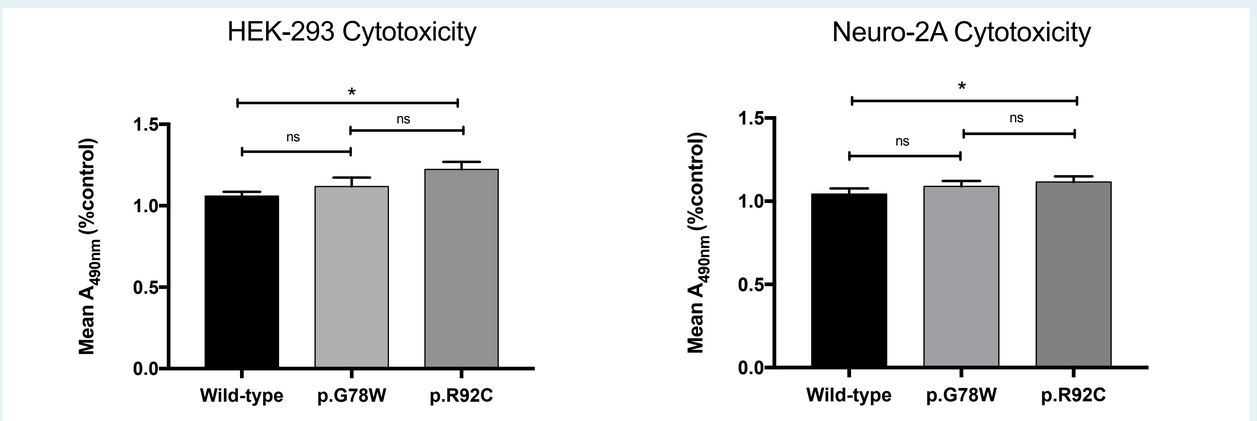

Fig. 3. p.R92C correlates with a significant increase in the cytotoxicity of HEK-293 and N2A cells. LDH assay shows a positive correlation between p.R92C expression and cytotoxicity of HEK-293 and N2A cells, relative to over-expression of the WT gene (n=6; ANOVA, p<0.05).

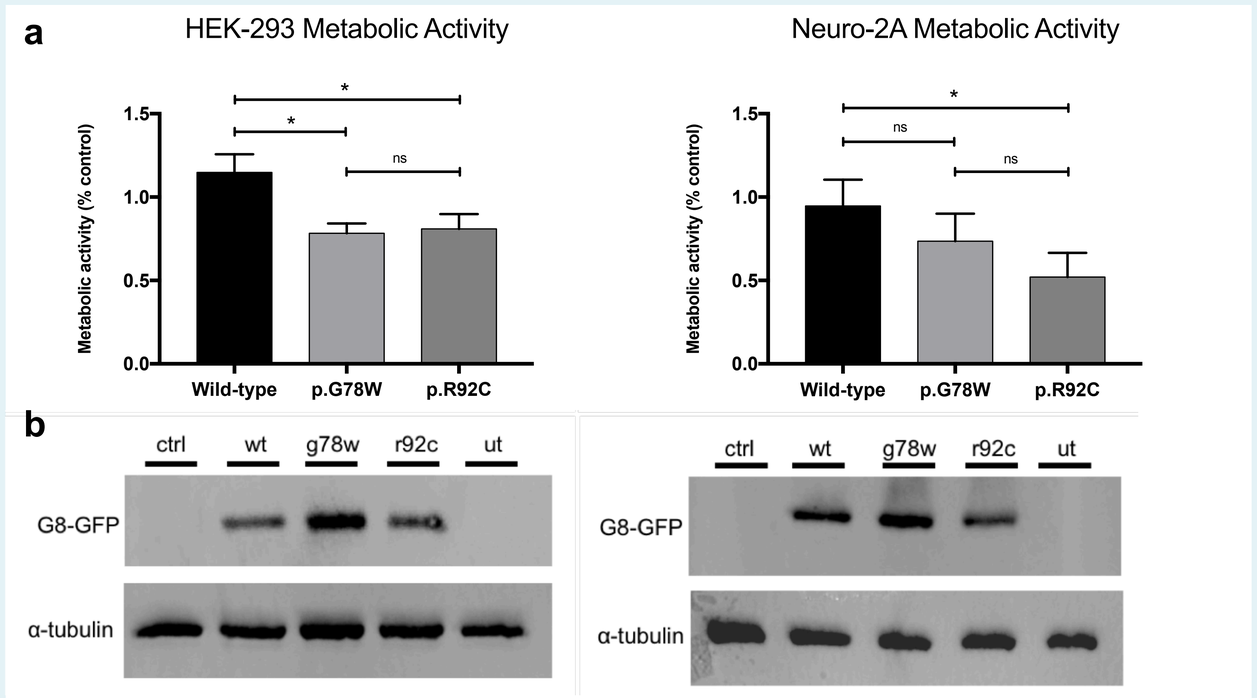

Fig. 4. p.G78W and p.R92C mutations correlate with a reduction in the proliferation rate of HEK-293 and N2A cells. (**a**) MTT assay suggests both mutations reduce cellular metabolism, relative to over-expression of the WT gene (n=6, ANOVA, p<0.05). (**b**) Immunoblots showing relative expression of WT and mutant protein.

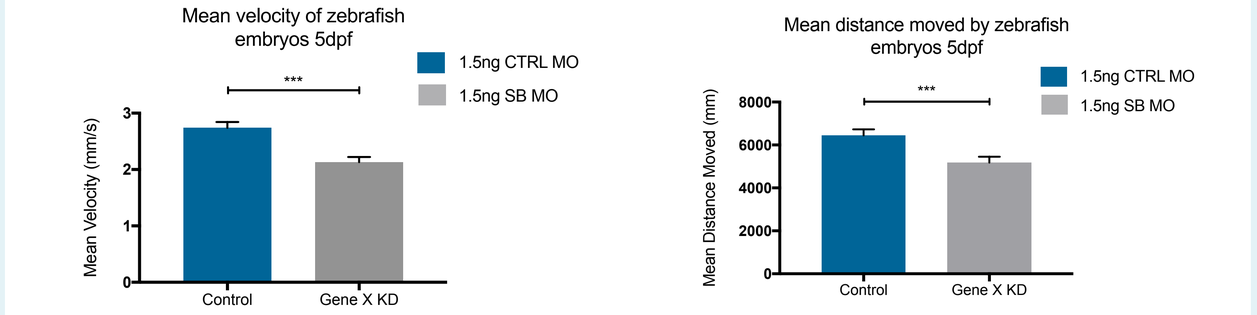

Fig. 5. Knockdown of gene X correlates with a reduction in the velocity and distance moved by zebrafish. Mean velocity (mm/s) and mean distance moved (mm) by zebrafish embryos (5dpf) injected with 1.5ng control morpholino and 1.5ng gene X-splice blocking morpholino. (n=3; un-paired *t*-test, p<0.005).

CONCLUSION

Protein X is poorly characterised but it contains a glycosyltransferase domain. We have shown that protein X is expressed in neurons and may localise to the Golgi network. Glycosyltransferase activity is associated with ganglioside synthesis which has been shown to be disrupted in ALS[1][2], however it has not been clear previously whether this was a cause or a consequence of disease. For the first time this work may show that dysfunction of ganglioside synthesis is an upstream cause of ALS.

1. Ariga, T. (2014). Pathogenic role of ganglioside metabolism in neurodegenerative diseases. J *Neurosci Res*, 2014. **92**(10): p. 1227-42.
2. Dodge, J. C., Treleaven, C. M., Pacheco, J., Cooper, S., ... Shihabuddin, L. S. (2015). Glycosphingolipids are modulators of disease pathogenesis in amyotrophic lateral sclerosis. *PNAS*. **112**(26): 8100-8105.