Antioxidant drugs reveal the potential for patient stratification in Motor Neurone Disease

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Introduction

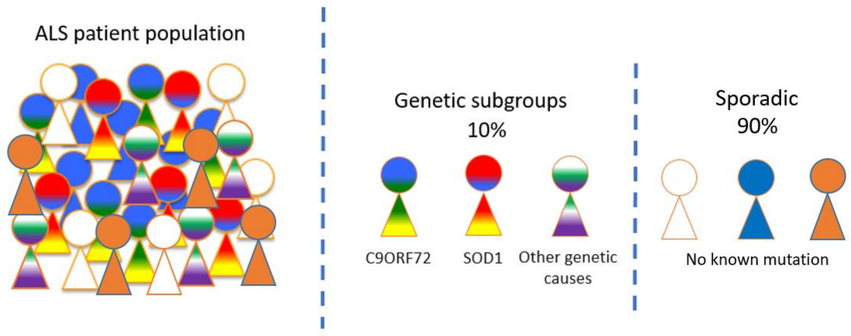
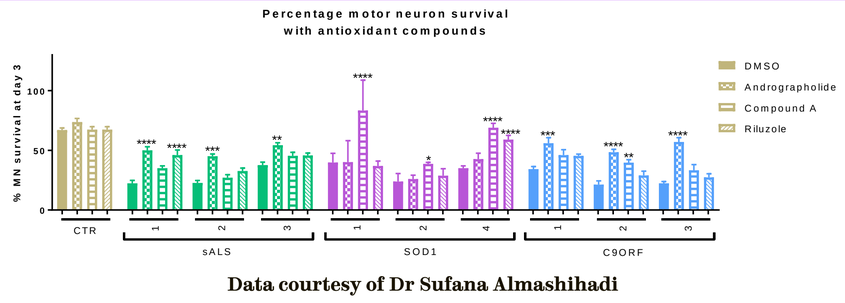


Figure 1. Many clinical trials investigating new ALS treatments have failed due to the vast heterogeneity of the patient population, resulting in compounds benefitting a small patient subgroup[1]. A previous study at SITraN outlined the potential for antioxidant compounds that had beneficial effects on specific subgroups of ALS patient astrocytes.

Different patient astrocytes react differently to the same antioxidant drugs



Data courtesy of Dr Sufana Almashihadi

Figure 2. Previous study investigating the beneficial effects of antioxidant compounds on specific subgroups of ALS patient astrocytes. Motor neurons were co-cultured with astrocytes pre-treated with 10uM of antioxidant compound or Riluzole and the percentage of motor neuron survival at day 3 was established. Motor neurons on control astrocytes had a 60-70% survival, while there was lower survival when co-cultured with the patient astrocyte lines. Different antioxidant compounds exerted beneficial effects on specific patient subgroups; Andrographolide significantly improved motor neuron survival in all C9ORF patient lines while Compound A* significantly improved survival in SOD1 patient lines. Sporadic lines reacted differently to different drugs.

RNA-sequencing analysis of patient astrocytes determines differences between subgroups

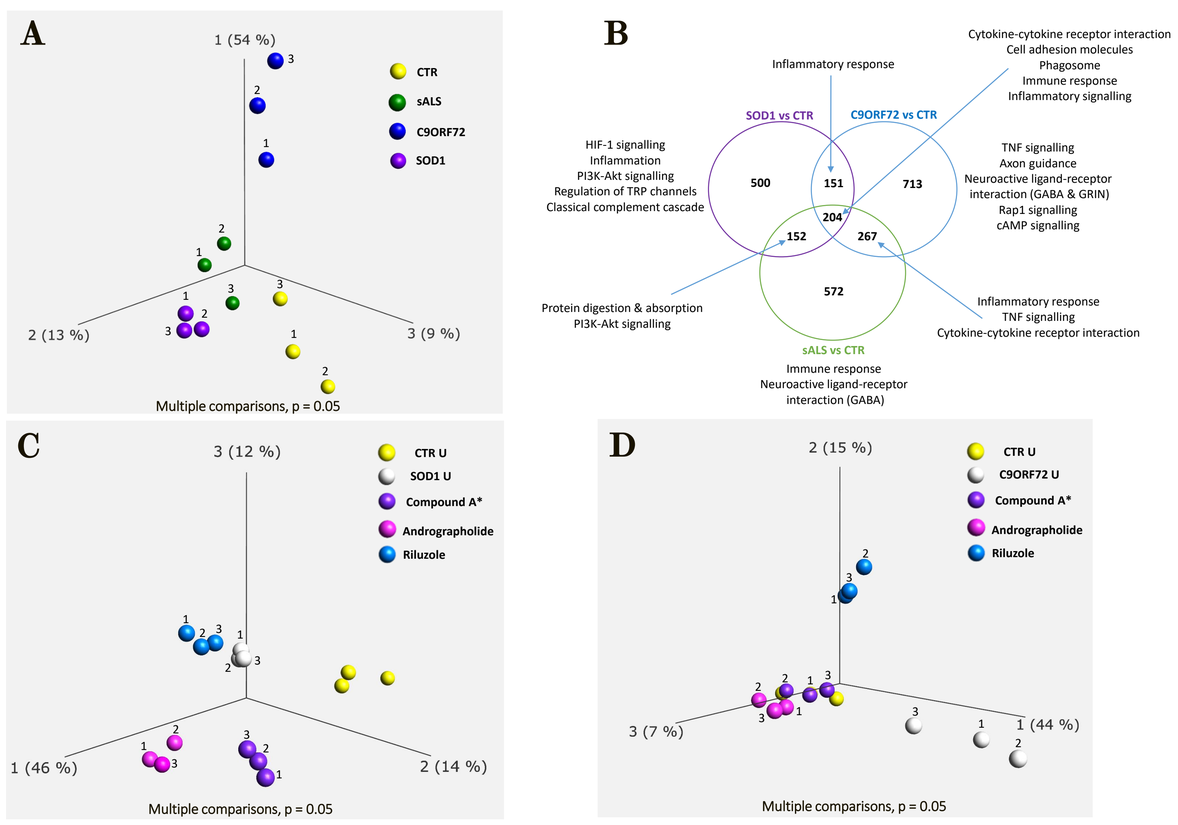


Figure 3. RNA-sequencing analysis of the translatome profile of the patient astrocyte lines compared to the control cell lines. **(A)** Principle Component Analysis (PCA) of the control and patient astrocyte lines. Genetic subgroups cluster together closely and separately from other subgroups, implying that there are large differences between controls and between patients. **(B)** KEGG pathway analysis of the RNA-seq data demonstrated differently regulated pathways in patient astrocytes over controls and these pathways were specific to the subgroup; large upregulation in pro-inflammatory pathways in SOD1 astrocytes while C9ORF72 pathways focused on transport. PCA analysis demonstrates that SOD1 **(C)** and C9ORF72 **(D)** patient astrocytes group closely when treated with the same compound and separate from the untreated group.

Conclusions

RNA-seq analysis of the astrocytes reflects the distinct grouping of patients in the ALS population and is able to identify pathways linked with ALS as well as identifying new pathways.
Misfolded SOD1 is characteristic of not only SOD1 and sporadic patients, but is also present in C9ORF72 patient-derived astrocytes which reflects previous studies in patient tissue[3].
Compound A* reduces the presence of misfolded SOD1 aggregates from the perinuclear space through mechanisms under investigation.
ALS patient astrocytes display alterations in the mitochondrial network compared to control astrocytes and Andrographolide treatment results in a significant shift in mitochondrial dynamics, suggesting the compound might induce mitophagy[4].

References

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4. Guo W, Sun Y, Liu W. et al (2014) Small molecule-driven mitophagy-mediated NLRP3 inflammasome inhibition is responsible for the prevention of colitis-associated cancer. Autophagy; 10(6):972-85.

Acknowledgements
We would like to thank the ALS patients that have donated their fibroblasts for this study (STH16573) and the MRC DiMeN DTP scholarship programme for their funding supporting CA. *Compound A is currently undergoing patent.

Objectives

To decipher genetic signatures that will discriminate between different patient groups as well as investigating drug response at a cellular level.

Methodology

Astrocytes were generated from three unaffected individuals and three different patient subgroups of ALS (n=3 per group) to identify the modes of action of Riluzole and two antioxidant drugs, Andrographolide and Compound A*. The effects of these compounds on common hallmarks of ALS pathology were assessed.

Compound A* treatment reduces the presence of misfolded SOD1 aggregates in ALS astrocytes

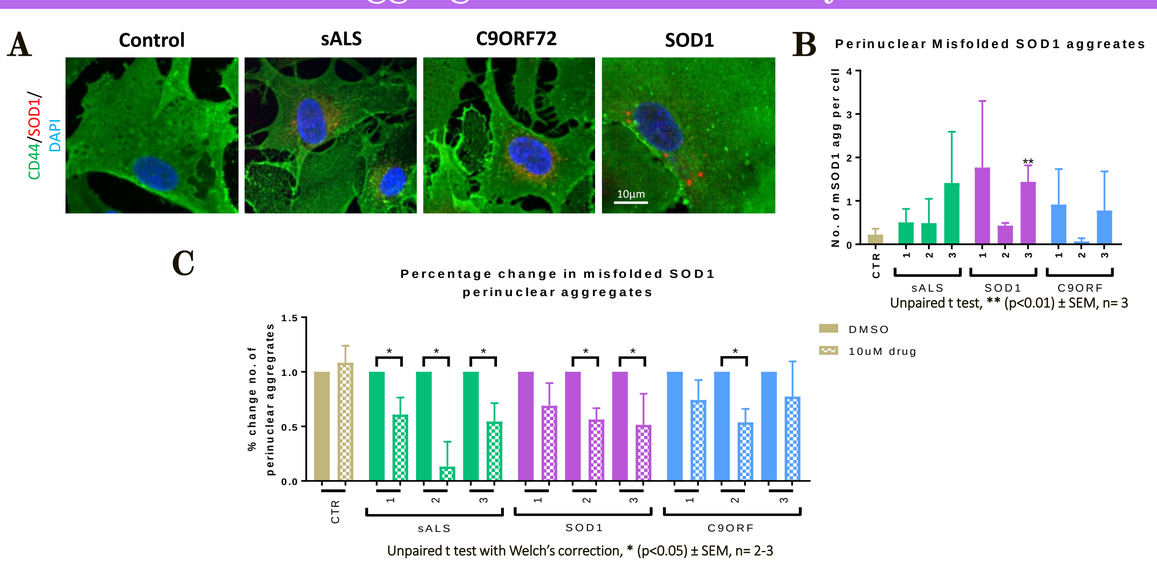

Figure 4. Characterisation of misfolded SOD1 protein within different astrocyte lines. **(A)** Visualisation of control and patient astrocytes using immunocytochemistry; misSOD1 (red), CD44 (green), Hoechst (blue). **(B)** MisSOD1 is a characteristic that is not exclusive to SOD1 patients; baseline levels of perinuclear misSOD1 show that the majority of patient cells have more aggregates than the control cells. **(C)** The percentage change of perinuclear misSOD1 aggregates from baseline levels after Compound A* treatment; there is a ~ 50-75% loss of misfolded SOD1 across all cell lines post-treatment.

Andrographolide treatment results in a shift in mitochondrial dynamics towards fusion

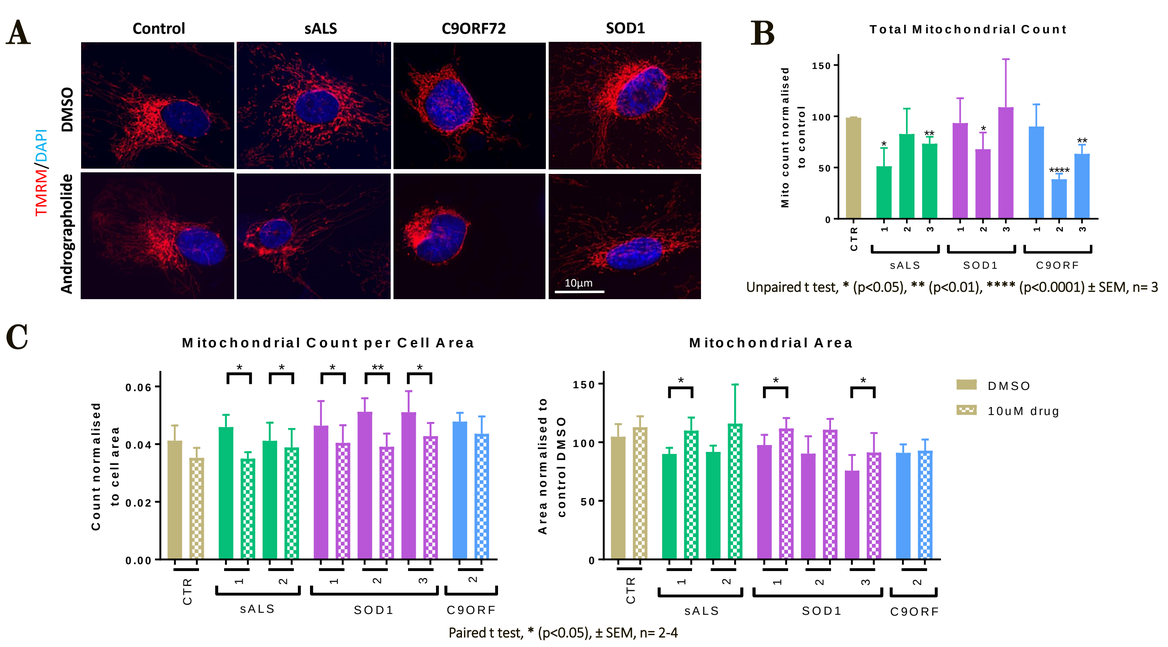

Figure 5. Investigating mitochondrial morphology and function using a mitochondrial membrane potential assay. **(A)** Visualisation of the mitochondria using a TMRM dye (red) and nucleus using Hoechst (blue). **(B)** The mitochondrial networks of each cell line differ slightly from another as shown by the mitochondrial count and area parameters. One of the SOD1 lines demonstrated severe mitochondrial fragmentation shown by significantly increased count, as previously reported in SOD1 motor neurons[2]. **(C)** When treated with Andrographolide, there is a significant decrease in mitochondrial count and increase in mitochondrial area which reflects the intense mitochondrial fusion among the majority of astrocyte lines.

Future work

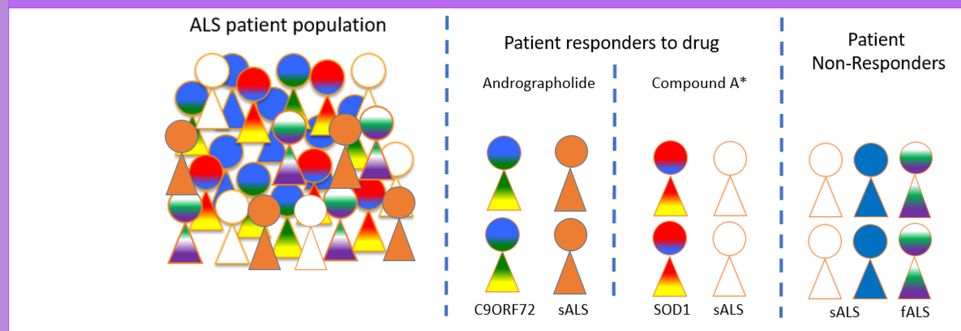

Figure 6. Astrocyte lines subjected to antioxidant compounds and Riluzole treatment have undergone RNA-sequencing analysis. This data, alongside the functional cellular assays, are likely to identify gene signatures for patient stratification as well as determining mechanism of action of compounds. We hope that we can drive the future of ALS patient treatment towards a more personalised approach.