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JOURNAL CLUB

Improving survival in amyotrophic lateral sclerosis: future treatments in a modern service

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Efforts to improve survival in amyotrophic lateral sclerosis (ALS) to date have yielded only modest results, leading to an historically nihilistic approach to management. In the meantime, genetic advances continue to provide new pathways for therapeutic opportunity and the ALS gene discovery curve is exponential. Indeed, the first paper discussed this month confirms the discovery of another new gene associated with ALS and provides further novel insights into the pathogenic mechanism.

A second paradigm shift in the understanding of the genetic contribution to ALS aetiology came with the discovery of the *C9orf72* hexanucleotide repeat expansion, reviewed in a previous journal club article in 2012. However, subsequent attempts to explain the mechanisms by which this and other genes cause disease and how they are regulated has been challenging. The second paper discussed this month investigates the association between methylation of the *C9orf72* promoter and disease severity.

Despite the rapid evolution of the understanding of ALS pathogenesis, effective disease-modifying therapies remain years away from clinical use. It therefore remains of paramount importance to investigate holistic methods of improving life expectancy. In the final paper discussed this month, Rooney et al. utilise the political divide between Northern Ireland and the Republic of Ireland to compare outcomes of two models of service delivery for ALS patients at a population level, with convincing results.

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Haploinsufficiency of *TBK1* causes familial ALS and fronto-temporal dementia

The advent of next generation sequencing techniques has recently led to an acceleration of the discovery of genetic variants associated with ALS, which has substantially improved understanding of its pathophysiology. Freischmidt et al. utilised whole-exome sequencing in combination with a statistical method shown to maximise sensitivity for detecting rare variants whilst maintaining specificity for identifying those that are functional. Two hundred and fifty-two familial and 1010 sporadic cases with 650 controls were recruited after negative screen for *SOD1* mutations and *C9orf72* expansions. A search for loss of function mutations identified seven variants of the gene *TBK1* that associated with familial ALS, confirming the results of a recent GWAS. However, targeted mutation analysis in sporadic cases found only three patients had one of the mutations, suggesting that they are far more important in familial ALS.

The study proceeded to analyse gene expression and protein function in mutation-carrying patient-derived cell lines to provide further insights into pathogenic mechanisms. It appears that haploinsufficiency (reduced expression of the abnormal allele) is the primary mechanism of disease in familial cases. This was based on the enrichment of loss of function mutations and the demonstration that four out of five mutations analysed created a premature STOP codon and resulted in a 50 % reduction in *TBK1* mRNA and protein levels. Further proteomic work also revealed that the disease-related part of the TBK1 protein is its CCD2 domain, which binds optineurin. Mutations in optineurin have been found in some cases of familial ALS, thus suggesting a common pathogenic pathway. Finally, although haploinsufficiency appears the most significant

mechanism, analysis of missense variants of *TBK1* also displayed the expected functional impairments of protein function, suggesting that these may be important, but with lower penetrance.

Comment. This study confirms that *TBK1* mutations cause 4 % of familial ALS cases, with 50 % of those identified also displaying elements of FTD. Whilst this accounts for a small proportion of familial cases, when taken together with the recent discovery by Cirulli et al. that *TBK1* is also enriched in sporadic ALS cases on GWAS, this mechanism may be relevant to many cases. The functional consequences of mutations in *TBK1* are also evaluated in substantial detail and provide a new potential therapeutic target pathway as compounds targeting TBK1 signalling have already been developed for treating cancer. There will undoubtedly be further variants discovered as genome sequencing technology extends out of the exome and into non-coding regions, which often display associations with disease, but by as yet undetermined mechanisms.

Freischmidt A et al (2015) Nat Neurosci 18(5):631–636.

DNA methylation slows effects of *C9orf72* mutations

This paper reports a study investigating the effects of methylation of the promoter sequence of ALS-associated gene *C9orf72*, as measured by neuroimaging, neuropsychological testing and neuropathological findings. Hexanucleotide repeat expansions of *C9orf72* are the commonest genetic abnormality found in familial and sporadic ALS-FTD spectrum disorder, but whether disease results from loss of function or pathological gain of function is not known. Methylation of DNA at CpG sites in the regulatory elements of genes is a well-established mechanism by which gene expression is regulated. Hypermethylation of a gene's promoter is associated with reduced expression, whilst the converse is true for hypomethylation, and the process appears to play an increasingly prominent role in neurodegenerative disorders.

The initial part of the study recruited 20 patients with pathological expansions of *C9orf72*, along with 25 controls. Methylation of the *C9orf72* promoter was measured using a well-recognised technique involving methylation-sensitive restriction enzymes and quantitative PCR. All patients and controls underwent volumetric brain MRI to measure grey matter density, followed by repeat imaging in 11 of the cohort after approximately 1 year. Cross-sectional and longitudinal analysis revealed that increased *C9orf72* promoter hypermethylation was found to associate with preserved grey matter density in the right hippocampus, right thalamus and left premotor cortex, suggesting a neuroprotective effect. Neuropsychological testing at the time found hypomethylation also associated with accelerated decline in verbal recall, a function attributed to the hippocampus. Neuropathological verification in an independent postmortem cohort of 35 patients with a *C9orf72* expansion showed that hypermethylation of the *C9orf72* promoter in the cerebellum protected against neuronal loss in the frontal cortex and hippocampus.

Comment. The multi-faceted approach of this study provides converging evidence that hypermethylation of the *C9orf72* promoter is protective against neuronal loss in patients carrying the pathogenic expansion. The numbers were relatively small and there was significant phenotypic heterogeneity; however, the authors make an attempt to account for this in post hoc analysis. The evidence is convincing enough to stimulate efforts to test the effects of increasing methylation of the *C9orf72* promoter. The challenge will be to achieve this in a sequence-specific manner, as methylating agents operate at a genome-wide level. New experimental methods utilise bacterial systems to direct methylating agents to their target loci, which could prove to be a productive therapeutic avenue. Peripheral blood *C9orf72* promoter methylation could also be a useful biomarker as it has been found to closely correlate with the methylation pattern in the frontal cortex, which could provide valuable prognostic information and inform treatment decisions. The rapidly expanding field of epigenetics is likely to reveal complex links between genotype and phenotype and may soon offer novel therapeutic opportunities.

McMillan CT et al (2015) Neurology 84:1622–1630.

A multidisciplinary clinic approach improves survival in ALS: a comparative study of ALS in Ireland and Northern Ireland

Comparing healthcare systems and models of service delivery is often extremely difficult due to confounding factors and variable data collection. Rooney et al. report an elegant population-based study comparing the care systems for ALS patients in the Republic of Ireland (RoI) and Northern Ireland (NI): two very similar countries with different healthcare systems. NI operates an ALS Care Network led by a Coordinator from a nursing background, with care provided by community professionals local to the patient. The RoI operates a centralised multidisciplinary (MDT) clinic structure led by a specialist neurologist, which provides care for up to 80 % of the patient population. The remaining 20 % of patients do not access the specialist MDT service, but instead attend general neurology clinics.

Registry data for all 719 ALS cases diagnosed between 2005 and 2010 were included in a survival analysis.

Survival from diagnosis was compared between RoI and NI, adjusting for confounders such as time from onset to diagnosis, age and medical interventions. Survival was significantly better in RoI compared to NI: 1.22 years compared with 0.98 years, respectively. Notably, patients attending a specialist MDT displayed a statistically significant survival benefit of 8 months compared with those in RoI and NI that did not.

Comment. In a condition with a life expectancy from onset to death of 3–5 years, an 8-month improvement in prognosis is substantial. The survival benefit appears independent of the use of interventions such as NIV or medication and the authors conclude that the benefit lies in timely complex decision-making and communication with community-based professionals. This may be in combination with an immeasurable holistic benefit like that shown by acute stroke units. The study is limited by the absence of analysis of the impact of socioeconomic factors and cognitive impairment as confounders, but still provides convincing evidence in support for the centralised specialist MDT model for managing ALS at a population level. Indeed, Northern Ireland has since changed its service to move in line with that of the Republic of Ireland, and perhaps this model should be universally deployed.

Rooney J et al (2015) J Neurol Neurosurg Psychiatry 86:496–501.