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Markers of cognitive resilience and a framework for investigating clinical heterogeneity in ALS[†].

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Short running title: Markers of cognitive resilience in ALS.

Abstract

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder. Despite the unifying pathological hallmark of TDP-43 proteinopathy, ALS is clinically a highly heterogeneous disease, and little is known about the underlying mechanisms driving this phenotypic diversity. In a recent issue of *The Journal of Pathology,* Banerjee *et al*. use region-specific transcriptomic profiling in *postmortem* brains from a deeply phenotyped clinical cohort of ALS patients to detect molecular signatures differentiating cognitively affected and unaffected patients. They identified differential expression of specific genes, including upregulation of pro-inflammatory IL-6 in the cognitively affected group and anti-inflammatory IL-1 in the cognitively unaffected group. They then utilised BaseScope™ *in situ* hybridisation and immunohistochemistry to validate upregulation of *NLRP3*, an activator of the inflammasome, in the cognitively affected group, and upregulation of *SIRT2*, an inhibitor of NLRP3, in the cognitively unaffected group. In summary, Banerjee *et al.* demonstrate the usefulness of combining a well curated clinical cohort with transcriptomic analysis of pathological samples to identify a perturbed pathway (*e.g.,* the inflammasome), offering opportunities for novel therapeutic targets in ALS.

Keywords: Amyotrophic lateral sclerosis, motor neuron disease, cognition, inflammasome, NLRP3, SIRT2, IL-1, IL-6.

Main Text

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder, with a lifetime risk of 1 in 300 people. It is well established that almost all people with ALS exhibit TDP-43 proteinopathy [1]. However, despite this unifying pathological feature, ALS is clinically a highly heterogeneous disease, with varying: sites of symptom onset (upper limb *vs* lower limb *vs* bulbar); involvement of clinical domains (upper motor neuron *vs* lower motor neuron *vs* cognitive); and rapidity of disease progression. The underlying disease mechanisms driving phenotypic diversity are poorly understood. Moreover, in clinical trials, people with ALS with diverse clinical phenotypes and pathological features are frequently grouped together, potentially masking the ability to adequately detect efficacy of a treatment intervention on a more clinically homogeneous patient subset [2].

In their study, Banerjee *et al.* elegantly demonstrate the usefulness of having a well curated, deeply phenotyped clinical cohort, which, when coupled with techniques able to detect altered molecular signatures in *postmortem* pathological samples, can be used to better understand complex ALS mechanistic pathways [3]. They focussed on understanding why 50% of people with ALS are cognitively unaffected. A previous study combining the analysis of cognitive function throughout disease and detailed *postmortem* investigations of the extra-motor brain regions, found that, although TDP-43 pathology was a very sensitive marker for cognitive impairment, there were also patients with similar levels of TDP-43 pathology that had shown no changes upon repeated cognitive testing [4]. What underlies this resilience to cognitive impairment? Banerjee *et al*. sought to discover a more specific neuropathological correlate of cognitive involvement than TDP-43 proteinopathy, with the aim of identifying differentially affected molecular pathways.

Validation of brain region-specific transcriptomics data necessitates the employment of complementary, spatially resolved techniques to confirm cell-type specific differences between cohorts. Indeed, spatial transcriptomic approaches have been used to interrogate ALS disease progression pathways by profiling both anatomically and temporally defined gene expression changes at the cellular level within *postmortem* spinal cord [5-7]. The recent development of BaseScope™, a high-resolution *in situ* hybridisation (ISH) technique, has been utilised to improve both the detection rates of *C9orf72* RNA foci [8] as well as the identification of dysregulated transcripts within neurons specifically associated with the *C9*-ALS clinical phenotype [9]. The high sensitivity of BaseScope™-probes designed to target specific splice variants has also led to its use in validating novel cryptic exon events within ALS and frontotemporal dementia patient brains [10,11].

Additionally, fluorescence-activated sorting (FACS) of cell populations enriched for disease-associated pathological signatures, *e.g.,* TDP-43 depletion, is another powerful transcriptomics method for delineating ALS-specific disease pathways in brain regions of interest [12]. Indeed, FACS has the potential to provide more in-depth information surrounding key molecular signatures underlying specific disease phenotypes and their relevance to particular cell types and pathological substrates. Robust clinical stratification followed by brain region-specific bulk or FACS purified cell population transcriptomics with combined spatially resolved *ISH* validation, represents an effective workflow for investigating phenotypic heterogeneity in ALS (Figure 1).

In their study, Banerjee and colleagues utilised BaseScope™ *in situ* hybridisation (ISH) to validate two gene targets identified as being differentially expressed (using the NanoString

nCounter neuropathology panel) in opposite directions between cognitively affected and unaffected ALS patients with similar levels of TDP-43 pathology. First, they confirmed differential upregulation of *NLRP3*, indicating activation of the inflammasome, in cognitively affected ALS individuals within both layer V cortical neurons and glial cells of pre-identified brain regions with extra-motor (clinical) correlates of cognitive dysfunction. They also elucidated a reciprocal change in *SIRT2*, a known neuronal-specific repressor of the inflammasome. *SIRT2* was found to be specifically upregulated in cognitively unaffected ALS patients within corresponding brain regions to *NLRP3*, and they thereby proposed its elevated expression to be a marker of cognitive resilience in ALS pathogenesis. The study adds to previous research suggesting that inappropriate activation of the inflammasome plays a role in neurodegenerative conditions, including ALS, and highlights that modulation of the inflammasome could be a therapeutic target in ALS patients, particularly in those with cognitive symptoms [13-16].

Future directions

Larger sample sizes of deeply phenotyped, clinically stratified *postmortem* brain and spinal cord cohorts will be necessary to further explore the disease mechanisms underpinning phenotypic heterogeneity (both cognitive and motor) within ALS patients. Dual immunohistochemistry (IHC)–ISH approaches with high spatial resolution may shed further light on both the identities of neuronal and glial subpopulations vulnerable to transcriptomic changes in particular clinical subtypes, as well as offering a platform for studying the interrelationship between TDP-43 proteinopathy and other potential disease correlates, such as the presence of aberrant splicing changes, in ALS.

In summary, the methodological pipeline used in this study provides a framework for future research projects focused on disentangling the complex relationships between clinical presentation, genetic status, and pathological findings, the clarity of which is essential to further the advancement of disease-appropriate biomarker and therapeutics development. Thus, collaborative efforts to coordinate the curation and maintenance of highly phenotyped clinical cohorts in specialist centres collecting *postmortem* data are crucial and invaluable to progressing neurodegenerative research.

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Author contributions

PRM and AB wrote the manuscript. All authors reviewed and critically revised the manuscript, and approved the final version for submission

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Figure legend

Figure 1. Methodological pipeline for delineating pathways underpinning ALS clinical heterogeneity. 1. ALS subjects are clinically stratified into distinct phenotypic subgroups according to their site of disease onset, rate of progression, upper (UMN) or lower motor neuron (LMN) predominance and cognitive status during life. 2. Clinico-anatomically relevant brain and spinal cord specimens from cohorts falling into two extremes of a selected phenotype (*e.g.,* cognitive involvement vs cognitive resilience as in Banerjee *et al.,* [3]) can then be selected for region-specific bulk-sequencing or pathologically resolved transcriptomic analysis using fluorescence-activated sorting (FACS). 3. Finally, transcriptomic findings of interest can be further dissected using immunohistochemistry and BaseScope™ *in situ* hybridisation.