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Allen, S. (2020) Understanding metabolic flexibility : a potential key to unlocking metabolic therapies in amyotrophic lateral sclerosis? Neural Regeneration Research, 15 (9). p. 1654. ISSN: 1673-5374

https://doi.org/10.4103/1673-5374.276333

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PERSPECTIVE

Understanding metabolic flexibility: a potential key to unlocking metabolic therapies in amyotrophic lateral sclerosis?

The role of metabolism in amyotrophic lateral sclerosis (ALS): ALS, is a predominantly sporadic, neurological disorder resulting in degeneration of both upper and lower motor neurons, resulting in the progressive failure of the neuromuscular system. The lifetime risk factor for developing ALS sits between 1 in 300 and 1 in 400. However, due to the speed of the disease course (2–3 years post-diagnosis) and the lack of effective therapies, ALS is still classified as a rare disease. It is well established that ALS affects energy generation not only in the central nervous system (CNS) but in the periphery (Vandoorne et al., 2018). However, the question of whether metabolic dysfunction is the primary cause of ALS or a secondary effect has been the topic of discussion in the field for many years. Metabolic dysfunction is observed in cellular models of the disease, early in the disease course in animal models and in both patient post-mortem tissues and biofluids. However, over the last 10–15 years, many of the ALS genes identified have been linked to RNA processing or function. These include TDP43 (TARDBP or TAR DNA binding protein 43 a key pathological marker of ALS), fused in sarcoma (FUS) and an intronic expansion in *C9orf72* which accounts for approximately 10% of all ALS and frontotemporal dementia cases, including a significant proportion of both familial and apparently sporadic cases. This leads to the question of whether RNA processing defects are the key pathogenic event in sporadic ALS and how this relates, if at all, to energy metabolism defects? Indeed, a recent publication from Vandoorne et al. (2019) indicated that induced pluripotent stem cell derived motor neurons with an ALS causing FUS mutation did not show metabolic dysfunction in spite of recapitulating other aspects of the disease (Guo et al., 2017). However, and as stated by the authors, their results do not discount the role of metabolic dysfunction in motor neuron dysfunction. Intriguing, the lack of metabolic dysfunction in these motor neurons potentially points to non-neuronal cells such as astrocytes, or muscle as the source of metabolic dysfunction in ALS (Dupuis et al., 2009; Allen et al., 2019a). A classical metabolomic approach has been taken by many researchers to try and uncover the role of energy dysfunction in ALS. Although it is clear our neuronal and non-neuronal models of ALS have a distinct metabolic profile compared to controls, a lack of consistency between studies have hampered the field in terms of metabolic biomarker identification. 15 years on from the first metabolomic study in ALS (Rozen et al., 2005) the field is still unclear regarding how the disease affects the many catabolic pathways that feed into the major energy generating systems in the cell. If we regard ALS as primarily a RNA processing/export disease for example, this could either directly or indirectly, affect the transcript levels or the splicing of multiple components within these catabolic pathways. Understanding how ALS affects global cellular catabolism would allow researchers to manipulate catabolism therapeutically or nutritionally to increase carbon flow into the glycolytic or mitochondrial energy generating pathways. If metabolic dysfunction is not the cause of ALS then it certainly is an early, important cause of the disease because it can affect disease progression rates (Steyn et al., 2018). Therefore, in depth understanding of the metabolic pathways and how they are affected in ALS could lead to therapies that slow down the disease progression and increase the quality of life of people with the disease.

Metabolic flexibility: In our laboratory we have used fibroblasts as an initial human derived model of ALS. The major criticism of this model is that it is not a CNS cell and that fibroblast toxicity is not observed in ALS. However, we and others have found that metabolically, fibroblasts recapitulate some of the pathogenic dysfunction observed in the CNS including metabolic dysfunction (Allen et al., 2019a). In our laboratory this led to the question, what can a fibroblast do metabolically that an astrocyte for example cannot? We found that fibroblasts isolated from superoxide dismutase-1 (SOD1) ALS patients had reduced mitochondrial capacity (Allen et al., 2014). However, unlike astrocytes and motor neurons (**Figure 1**), fibroblasts were able to upregulate glycolytic flux to maintain energy production. This raised the possibility that inherent metabolic flexibility may play a protective role against ALS and may be linked to polygenicity and/or CNS specific disease modifying factors. Rather than ALS being solely monogenic in nature, the disease itself, what age it manifests and the speed of progression may be linked to a number of interacting rare variants, which in combination with environmental factors may act as CNS disease modifiers which influence disease onset and progression (Al-Chalabi et al., 2014). The result of these interactions may be patient group or even patient to patient specific and may influence how patients respond to for example, dysfunction in RNA processing, drugs or nutritional intervention (Allen et al., 2019a), which could impact on the overall effectiveness of dietary or therapeutic interventions. These potential disease modifying interactions could be responsible for the disease heterogeneity observed in ALS patients. It is clear that different genetic backgrounds or levels of gene overexpression can affect factors such as disease onset or survival in animal models of ALS. However, it could be argued that patient heterogeneity is not recapitulated to the same extent, which contributes to the lack of therapeutic translation observed in the ALS field between animal models and patients. Therefore, a lack of a successful translatable animal model makes the use of human models of disease such as fibroblasts all the more relevant, especially as they can be readily reprogrammed into inducible neuronal progenitor cells (Meyer et al., 2014). Induced neuronal progenitor cell reprogramming overcomes the challenging phenotypic inconsistency of clonal variation often observed in induced pluripotent stem cells. Furthermore, the reprogramming does not reset the epigenetic state of the fibroblasts so inherent aging phenotypes are retained which reduces the reliance on post-mortem tissue (Mertens et al., 2015).

Phenotypic metabolic screening: The idea of investigating flexibility in ALS originated from our early laboratory work in fibroblasts in 2014 and came to fruition in two papers in 2019 (Allen et al., 2019a, b). In these studies we utilized phenotypic metabolic screening technology developed by Biolog Inc. (Hayward CA. USA), which is an alternative to the classic metabolomic approach and had not been previously used in the ALS field. The technology generates a kinetic metabolic profile of the cell by removing glucose and supplementing in the case of their PM-M1 carbohydrate plate, with energy substrates from 91 different points in the catabolic pathways simultaneously. This global profiling approach where cells are starved of glucose and fed individual metabolic substrates allowed us to, for the first time in the ALS field, directly measure metabolic flexibility in human models of *C9orf72* and sporadic ALS. Moreover, using fibroblast-derived induced neuronal progenitor cells differentiated into iAstrocytes we were able to assess how the metabolic profile and metabolic flexibility was altered by the reprogramming process. We showed distinct metabolic profiles between fibroblasts and iAstrocytes and that patient fibroblasts maintained

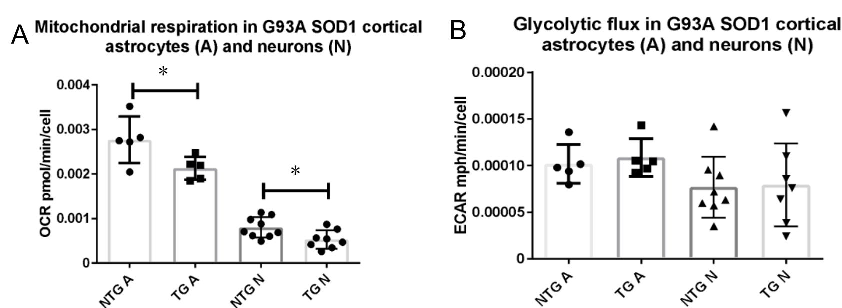

Figure 1 Cortical astrocytes at p1 and cortical neurons at E15 were isolated from C75/BL-6 G93A SOD1 mice and cultured.

15,000 astrocytes or 75,000 neurons per well were analyzed for their mitochondrial respiration (A) and glycolytic flux (B) using an XF24 bioanalyser as previously described (Allen et al., 2019a). Data presented as one data point per independent biological replicate (each data point being an average of 3–4 wells of a 24-well plate), data presented with mean and standard deviation. $*P \le 0.05$, using parametric *t*-test analysis. ECAR: Extracellular acidification rate; NTG: non-transgenic; OCR: oxygen consumption rate; SOD1: superoxide-dismutase-1; TG: transgenic.

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their metabolic flexibility compared to controls whilst metabolic flexibility was reduced in *C9orf72* and sporadic ALS iAstrocytes which contributed to starvation induced toxicity. Using this global profiling approach combined with principal component analysis and heat mapping, we were able to identify the catabolic pathways that were altered between controls and ALS cases in iAstrocytes. We found dysfunction in catabolic pathways that had not been implicated in ALS before including nucleoside, glycogen and fructose catabolism. Probing of the enzymes/proteins in these pathways elucidated new targets for both *C9orf72* and sporadic ALS including adenosine deaminase, glycogen phosphorylase, phosphoglucomutase and glyoxalase-1 (Allen et al., 2019a, b). All of these pathways contributed to a loss of metabolic flexibility in the ALS iAstrocytes. However, a number of primarily mitochondrial energy substrates that were reduced in the ALS iAstrocytes showed control level of enzymes involved in their catabolism. So we asked the question, does membrane transport of energy substrates contribute to metabolic flexibility? We therefore utilized a newly established technological advancement from Biolog Inc. which probes for mitochondrial function by saponin treating cells and feeding the cells a variety of cytosolic and mitochondrial substrates. We found that those mitochondrial energy substrates that were affected in the whole cell screen were unaffected in saponin treated cells and that *C9orf72* iAstrocytes had alerted saponin sensitivity. As saponin functions by binding cholesterol, our results suggested that cholesterol levels may be altered in *C9orf72* ALS which would affect membrane lipid structures, membrane transport levels and therefore, energy substrate membrane transport. These results showed that both efficient catabolism and membrane transport of energy substrates are crucial for effective metabolic flexibility in the CNS. Moreover, disruption to these processes leads to toxicity in the event of disrupted metabolic fuel supplies to the glycolytic and mitochondrial energy generation pathways.

Nutritional supplementation in ALS: Nutritional supplementation based on a sound understanding of how ALS affects the catabolic pathways has the potential to, if not cure the disease, have a significant impact on disease progression and quality of life. A number of trials are in progress including the HighCALS trial (RP-PG-1016-20006), which seeks to increase calorie intake in ALS patients and a high fat supplement trial in Europe (NCT02306590). This aims to give ALS patients a high fat diet which has been shown to be beneficial in the SOD1 mouse model of the disease. Only time will tell whether they are effective and in what subset of patients. Recently, inosine has emerged as a potential nutritional supplement in the ALS field, based on previously encouraging studies of inosine as a potential source or the antioxidant urate/uric acid in Parkinson's disease. However, a recent SURE-PD3, Phase III clinical trial in Parkinson's disease was halted due to a lack of efficacy and it remains to be seen how an inosine safety study in the United States (SURE-ALS2) will pan out. In our *in vitro* study (Allen et al., 2019a) we found that inosine supplementation in ALS iAstrocytes led to an increase in uric acid levels in all cases. However, inosine supplementation was not bioenergetically beneficial in all ALS case derived iAstrocytes. Intriguing, only those ALS iAstrocytes which responded bioenergetically to inosine became less toxic towards motor neurons in co-culture compared to those that did not respond bioenergetically. Therefore, elucidating the correct mechanism of action of any nutritional intervention is key before embarking on clinical trials. As is the ability to sub-classify patients, not based on ALS as a monogenic disease but based on ALS as a polygenic, multivariate disease with modifying factors that influence metabolic flexibility and the response to nutritional intervention or therapeutic intervention. This is a real benefit of our induced neuronal progenitor cell model of ALS, not only do they retain their aging phenotype, they are genetically distinct and unlike the homogenous animal models, recapitulate the ALS case to case heterogeneity observed in clinical trials, which negatively affect statistically positive survival outcomes.

In short, sub-classifying ALS patients based on their genetically influenced cellular response to the disease (which ties in with metabolic flexibility) or their response to therapeutic intervention, has the potential to open the possibility of designing patient specific therapeutic approaches to improve prognosis for people with ALS. Taking a phenotypic metabolic functional approach in patient-derived cells enables researchers to functionally metabolically fingerprint complex sporadic diseases such as ALS. This "metabotyping" bridges the gap between environmental influences on the genome and the heterogenic functional outcomes which has so far reduced our translational efficacy in this devastating neurological disease.

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Received: *November 26, 2019*
Peer review started: *November 30, 2019*
Accepted: *January 9, 2020*
Published online: *February 28, 2020*

doi: *10.4103/1673-5374.276333*
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Open peer reviewers: *Sagar Gaikwad, University of Texas Medical Branch at Galveston, USA; Rodolfo Gabriel Gatto, University of Illinois at Chicago, USA.*
Additional file: *Open peer review reports 1 and 2.*

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P-Reviewers: Gaikwad S, Gatto RG; C-Editors: Zhao M, Li JY; T-Editor: Jia Y