Metformin treatment of the C9orf72 ALS/FTD mouse: Almost too good for words

Michael Rosbash a,b,1

PNAS | August 18, 2020 | vol. 117 | no. 33 | 19627–19628

The regulation of protein synthesis is critically important for the normal development and function of the brain. Protein synthesis misregulation accompanies and in some cases underlies a diverse set of developmental and neurodegenerative diseases. They include Down syndrome, fragile X syndrome, Alzheimer’s disease, and amyotrophic lateral sclerosis (ALS) (1). Although the study by Zu et al. (2) is focused on C9orf72 ALS and frontotemporal dementia (FTD), the work has implications for a much larger family of more than 50 microsatellite expansions diseases, which include Huntington disease, myotonic dystrophy, and a number of spinocerebellar ataxias (3).

Microsatellite expansion diseases are caused when short stretches of repetitive DNA (e.g., CAG•CTG; CCTG•CAGG and G4C2•G2C4) located in protein coding or noncoding gene regions increase in length beyond a certain threshold. For C9orf72 ALS/FTD and other expansion diseases, these mutations result in a variety of downstream consequences, including the expression and accumulation of sense and antisense expansion transcripts. These RNAs can cause RNA toxicity as well as the sequestration of RNA binding proteins (4) and also serve as templates for the production of toxic proteins expressed from both sense and antisense expansion transcripts. PKR modulation is also more potent than stimulation of ER stress using thapsigargin, which works via PERK, or than addition of the ISR inhibitor.

Metformin is an inexpensive and widely prescribed drug, principally for type 2 diabetes. It has been in clinical use for more than 60 y and has a remarkable safety profile. Although a positive impact of metformin on neurodegenerative disease models, including dominant repeat expansion models, is not without precedent (12–16), the exciting feature of this paper is the intersection of its mechanistic advances and the impressive benefit afforded by metformin in ameliorating the effects of C9orf72-mediated ALS (Fig. 1).

The data show that metformin treatment reduces PKR phosphorylation and RAN protein levels. The paper convincingly shows that PKR activation by phosphorylation is more generally central to disease and leads to enhanced levels of RAN proteins. This is almost certainly principally due to their enhanced translation, in part via eIF2α phosphorylation by PKR. Metformin also improves behavior and decreases neuroinflammation as well as motor neuron loss in C9-BAC transgenic mice.

*Howard Hughes Medical Institute, Brandeis University, Waltham, MA 02453, and aDepartment of Biology, Brandeis University, Waltham, MA 02453

Author contributions: M.R. wrote the paper.

The author declares no competing interest.

This open access article is distributed under Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND).

See companion article, “Metformin inhibits RAN translation through PKR pathway and mitigates disease in C9orf72 ALS/FTD mice,” 10.1073/pnas.2005748117.
A key question for the future is how does metformin have such a potent effect on C9orf72-mediated ALS. Is it solely due to a reduction in RAN protein levels, and is this through a direct effect on PKR? A direct effect predicts that addition of metformin to a tissue culture model with PKR already phosphorylated will rapidly reverse this phosphorylation. An even stronger prediction is that metformin will affect an in vitro PKR phosphorylation assay.

Based on current knowledge however it is possible that metformin works indirectly and chronically to reduce p-PKR and RAN protein levels. Metformin up-regulates AMPK and directly inhibits mitochondrial complex I; the former is probably a consequence of the latter. Is this the direct target relevant to its therapeutic effect on the tissue of the latter. Is this the direct target relevant to its inhibits mitochondrial complex I; the former is probably a consequence of the latter. Is this the direct target relevant to its therapeutic effect on the C9orf72 mouse model of ALS? Perhaps the metformin sensitivity of this model could be examined in a genetic background resistant to the impact of metformin on mitochondrial complex I (17). Although making the appropriate mouse strain might not be straightforward, it would be striking were PKR and the disease still metformin-sensitive in this background. Such a result would strongly indicate the existence of an additional metformin target relevant to C9orf72-mediated ALS.

Metformin is known to have broad anti-inflammatory effects, many of which are due to chronic immune system modulation (12–14). Although the tissue culture experiments indicate that the drug can act directly on PKR-expressing cells, it is more difficult to show how the drug reduces RAN protein and p-PKR levels in the nervous system. Perhaps future genetic models can alter neurons or glia for in vivo studies, or a neuron/glia in vitro C9orf72 model can be created.

In any case, repeat expansion diseases with RAN proteins keep increasing in number; there are currently 50 and counting (4). Given the central role of stress pathways in regulating RAN translation, therapeutic approaches that target these central players may have efficacy across the entire family of diseases, indicating that metformin should be investigated as a potential therapy in a much larger group of microsatellite diseases. Metformin may have even broader applicability—from aging to COVID-19 (15, 16). It is hard to imagine an approved drug with more promise and a comparable quality:price ratio. More information is certainly around the corner.

1. S. Wiebe, A. Nagpal, N. Sonenberg, Dysregulated translational control in brain disorders: From genes to behavior. Curr. Opin. Genet. Dev. 65, 34–41 (2020).
2. T. Zu et al., Metformin inhibits RAN translation through PKR pathway and mitigates disease in C9orf72 ALS/FTD mice. Proc. Natl. Acad. Sci. U.S.A. 117, 18591–18599 (2020).
3. L. Nguyen, J. D. Cleary, L. P. W. Ranum, Repeat-associated non-ATG translation: Molecular mechanisms and contribution to neurological disease. Annu. Rev. Neurosci. 42, 227–247 (2019).
4. L. J. Szajdler, M. S. Swanson, Short tandem repeat expansions and RNA-mediated pathogenesis in myotonic dystrophy. Int. J. Mol. Sci. 20, 3365 (2019).
5. T. Zu et al., Non-ATG-initiated translation directed by microsatellite expansions. Proc. Natl. Acad. Sci. U.S.A. 108, 260–265 (2011).
6. T. Zu et al., RAN proteins and RNA foci from antisense transcripts in C9ORF72 ALS and frontotemporal dementia. Proc. Natl. Acad. Sci. U.S.A. 110, E4968–E4977 (2013).
7. K. Mori et al., The C9orf72 GGGGCC repeat is translated into aggregating dipeptide-repeat proteins in FTLD/ALS. Science 339, 1335–1338 (2013).
8. P. E. Ash et al., Unconventional translation of C9ORF72 GGGGCC expansion generates insoluble polypeptides specific to c9FTD/ALS. Neuron 77, 639–646 (2013).
9. K. M. Green et al., RAN translation at C9orf72-associated repeat expansions is selectively enhanced by the integrated stress response. Nat. Commun. 8, 2005 (2017).
10. W. Cheng et al., C9orf72 GGGGCC repeat-associated non-AUG translation is upregulated by stress through elf2α phosphorylation. Nat. Commun. 9, 51 (2018).
11. B. Tian et al., Expanded CUG repeat RNAs form hairpins that activate the double-stranded RNA-dependent protein kinase PKR. RNA 6, 79–87 (2000).
12. W. P. Black, Glasgow Obstetrical and Gynaecological Society Centenary 1885-1985. Scott. Med. J. 33, 378–380 (1988).
13. Y. Yin et al., Normalization of CD4+ T cell metabolism reverses lupus. Sci. Transl. Med. 7, 274ra18 (2015).
14. A. Singhal et al., Metformin as adjunct antituberculosis therapy. Sci. Transl. Med. 6, 263ra159 (2014).
15. A. S. Kulkarni, S. Gubbi, N. Barzilai, Benefits of metformin in attenuating the hallmarks of aging. Cell Metab. 32, 15–30 (2020).
16. P. Luo et al., Metformin treatment was associated with decreased mortality in COVID-19 patients with diabetes in a retrospective analysis. Am. J. Trop. Med. Hyg. 103, 69–72 (2020).
17. G. S. McElroy et al., NAD+ regeneration rescues lifespan, but not ataxia, in a mouse model of brain mitochondrial complex I dysfunction. Cell Metab., 10.1016/j.cmet.2020.06.003 (2020).