Metformin decreases RAN proteins and improves behavioral phenotypes in SCA8 BAC mice.

Lisa E.L. Romano[1], Setsuki Tsukagoshi[1], Emily Davey[1], Ramadan Ajredini[1], Tala Ortiz[1], Camille Preston[1], Elaine Ames[1], Monica Banez Coronel[1], Tao Zu[1], Eric T. Wang[1], Laura P.W. Ranum[1].

[1]University of Florida, Center for NeuroGenetics, College of Medicine, Department of Molecular Genetics and Microbiology, Gainesville, Florida

CTG•CAG expansion mutations cause several neurodegenerative and neuromuscular diseases, including myotonic dystrophy type1 (DM1) and multiple forms spinocerebellar ataxia (SCA). In SCA8, the expansion mutation is located in the overlapping ATXN8/ATXN8OS genes. Repeat-associated non-AUG (RAN) translation, now reported in 11+ diseases, including SCA8, is highly regulated by the double-stranded RNA-dependent protein kinase (PKR) pathway. Metformin, an FDA-approved drug and novel PKR inhibitor, substantially reduces RAN proteins in C9orf72 ALS/FTD BAC mouse model. Immunohistochemistry was used to test whether all six predicted RAN proteins accumulate in SCA8 human autopsy brains. SCA8-BAC transgenic mice, which express ATXN8/ATXN8OS using the endogenous human promoters, were used to test the effects of metformin on RAN protein reduction, behavioral phenotypes (DigiGait, rotarod, open field), and histopathological phenotypes. RNA sequencing was performed in the cerebellum to compare sense and antisense RNA levels and study splicing abnormalities in animals treated with or without metformin.IHC shows that sense and antisense RAN proteins accumulate in cerebellum from SCA8 (n>5) but not control (n>5) autopsy cases. Metformin-treated SCA8 mice showed improved rotarod (n>15/group, p=0.0017) and DigiGait (brake, n>15/group, p=0.002) performance compared to untreated SCA8 mice and non-transgenic littermates. IHC studies showed a dramatic reduction in neuroinflammatory markers (GFAP and Iba-1). RAN proteins are significantly reduced, while sense and antisense RNA levels are unchanged. SCA8 mice cerebellum presents splicing abnormalities that are partially rescued by metformin treatment.Most therapeutic strategies for repeat expansion disorders focus on targeting only the sense transcripts, neglecting the potential contribution of the antisense transcripts and antisense RAN proteins, which are now known to accumulate across the polyGln SCAs. These data show that the FDA-approved drug metformin reduces RAN protein load and improves behavior in SCA8 mice. Metformin is also able to partially rescue splicing abnormalities in SCA8 mice. If effective, it could be rapidly moved into clinical trials to test its efficacy as a safe and affordable treatment for patients with this devastating disorder.