



Splicing misregulation leads to metabolic vulnerability: vitamin B12 as a candidate therapy for PUF60-related Verheij syndrome

Jonathan Kölschbach¹, Hormos Dasfari^{1, 2, 3}, Emily Baum^{1, 2, 3}, Anna Lührke¹, Chun Kew⁴, Ivan Đikić⁴, Wenming Huang^{1, 5}, Adam Antebi^{1, 2} ¹Max Planck Institute for Biology of Ageing, Cologne, Germany, ²Cologne Excellence Cluster on Aging and Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany, ³Department of Pediatrics, Center for Rare Diseases, Faculty of Medicine and University Hospital Cologne, Cologne, Germany, ⁴Buchmann Institute for Molecular Life Sciences, Institute of Biochemistry II, Faculty of Medicine, Goethe University, Frankfurt, Germany, ⁵Institute of Clinical Genetics and Genomic Medicine, University Hospital of Würzburg, Würzburg, Germany

Background: mRNA splicing shapes proteome diversity and cellular state, and its disruption underlies a growing family of spliceosomopathies whose pathomechanisms remain poorly defined. Verheij syndrome, a rare multisystem disorder of developmental retardation and short stature, arises from mutations in the core splicing factor PUF60. How mis-splicing produces this disease, and whether it can be mitigated, has remained unknown. **Method:** We modeled PUF60 deficiency in *Caenorhabditis elegans* by introducing a hypomorphic mutation into the sole ortholog *rnp-6* or inducibly depleting the protein. Mechanistic studies included unbiased bacterial and mutagenesis screens, transcriptome-wide splicing analysis, metabolomics, lipidomics, and validation in PUF60-depleted human cells and patient plasma. **Results:** In *C. elegans*, RNP-6/PUF60 deficiency resulted in mis-spliced genes governing one-carbon and phospholipid metabolism, lowering methylation potential (SAM/SAH ratio) and depleting phosphatidylcholine. These perturbations activated the integrated stress response and suppressed mTORC1, delaying growth. Two independent screens converged on a single corrective input: vitamin B12. Supplementation of vitamin B12 restored SAM-dependent phospholipid remodeling and mTORC1 activity and rescued Verheij-like phenotypes. Mechanistically, intron retention in the transcription factor *nhr114/HNF4* emerged as a proximal disease driver. Forcing retention reproduced the disease state, intron deletion relieved the defects. Depleting another splicing factor, PRP-19, recapitulated the same metabolic disruption, and PUF60-deficient human cells and patient plasma showed parallel dysregulation of one-carbon and lipid metabolism. **Conclusion:** Mis-splicing of a metabolic transcription factor links spliceosomal dysfunction to disrupted methylation and phospholipid metabolism. Vitamin B12 supplementation reverses metabolic phenotypes to restore growth. Our findings suggest one-carbon metabolism

as a conserved vulnerability across spliceosomopathies, and warrant further investigation of vitamin B12 as a therapeutic strategy for spliceosomopathies.