Repeat associated non-AUG translation is a common pathogenic mechanism across the polyglutamine ataxias

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Repeat associated non-AUG (RAN) proteins have been reported in thirteen repeat expansion disorders. In *C9orf72*-ALS/FTD, RAN proteins have been shown to be important disease drivers. In spinocerebellar ataxia type 8 and Huntington disease, RAN protein aggregates increase with disease severity. Determining if RAN protein pathology occurs across the CAG•CTG polyglutamine spinocerebellar ataxias (CAG-SCAs) is critical for understanding the mechanisms of these diseases. Using newly developed α-polySer, α-polyLeu and locus-specific antibodies against unique C-terminal regions of each polySer and polyLeu RAN protein expressed from SCA1, SCA2, SCA3, SCA6 or SCA7 expansions mutations, we conducted immunostaining in SCA1,2,3,6&7 human postmortem tissue and SCA1 and SCA3 mouse brains. Sense polySer and antisense polyLeu RAN proteins show robust accumulation in SCA1, SCA2, SCA3, SCA6 and SCA7 throughout the most affected cerebellum and brainstem regions. In less affected frontal cortex, RAN protein aggregates are rare. Cell culture experiments show that CAG-SCA polySer and polyLeu RAN proteins are toxic to neural cells; RAN protein aggregates are ubiquitin-positive; cause autophagic dysfunction; and decreasing RAN proteins levels with metformin reduces cytotoxicity. In SCA3-YAC-84Q mice, cerebellar and pontine RAN protein aggregates increase with age. Pcp2-ATXN1^{82Q} SCA1 mice designed to express polyGln-expanded-ATXN1 in Purkinje cells, show prominent RAN protein aggregates throughout the cerebellum. Mice with disrupted ATXN1^{82Q}:capicua binding show improved phenotypes and markedly reduced RAN protein aggregates. These data support a pathogenic role of RAN proteins across the CAG-SCAs and highlight the need to understand their role in disease and evaluate therapeutic strategies targeting both sense and antisense transcripts.