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Double the Trouble: Bidirectional Expression of the SCA8 CAG/CTG Expansion Mutation—Evidence for RNA and Protein Gain of Function Effects

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Microsatellite expansions cause a number of dominantly-inherited neurological diseases including myotonic dystrophy (DM1 and DM2), Huntington's disease (HD and HDL2) and several forms of spinocerebellar ataxia (SCA). Expansions located in coding-regions cause dominant protein gain-of-function effects and non-coding expansions (DM and DM2) produce toxic RNA gain-of-function effects that in muscle have been shown to alter RNA splicing activities of MBNL and CELF proteins. We previously reported that a $(CTG)_n$ expansion causes spinocerebellar ataxia type 8 (SCA8)[1)]. Because the SCA8 expansion is transcribed, alternatively spliced, and polyadenylated in the CTG orientation we initially proposed that SCA8 is caused by an RNA gain-of-function mechanism similar to myotonic dystrophy. To elucidate the molecular events that cause SCA8, we developed a BAC transgenic mouse model in which the full length human SCA8 gene is expressed using its endogenous promoter[2)]. $(CTG)_{116}$ expansion, but not $(CTG)_{11}$ control lines, develop a progressive neurological phenotype and a loss of cerebellar cortical inhibition. Surprisingly, we found 1C2-intranuclear inclusions in Purkinje cells in SCA8 expansion mice and human SCA8 autopsy tissue result from translation of a nearly pure polyglutamine protein encoded on a previously unidentified anti-parallel transcript spanning the repeat in the CAG direction. The neurological phenotype found in the SCA8 BAC expansion lines but not BAC control lines demonstrates the pathogenicity of the $(CTG・CAG)_n$ expansion.

We discussed three lines of evidence that SCA8 CUG^{exp} transcripts cause RNA gain of function effects in the CNS[3)]. First, we demonstrate SCA8 CUG^{exp} transcripts form ribonuclear inclusions that co-localize with MBNL1. Second, we show that genetic loss of *Mbnl1* enhances motor coordination deficits in SCA8 mice. Third we show the *GABA-A transporter-4* (*Gabt4*) gene, which is dramatically upregulated in SCA8, is a misregulated MBNL/CELF splicing target. These data demonstrate for the first time that CUG^{exp} transcripts dysregulate MBNL/CELF regulated pathways in the brain and provide mechanistic insight into the CNS effects of other CUG^{exp} disorders (DM, HDL2). While functional evidence for RNA gain-of-function effects is presented here, the additional discovery of intranuclear polyglutamine inclusions in SCA8 suggests disease pathogenesis is mediated by toxic gain-of-function mechanisms at both the protein and RNA levels. Additionally, the growing number of bidirectionally-expressed genes in the genome suggests unrecognized CUG^{exp} RNAs contribute to some of the polyglutamine CAG・CTG disorders Please see Daughters et al., Plos Genetics.

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