Are Polyglutamine Diseases Expanding?

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It remains a matter of speculation as to whether the sense CUG-containing RNA and/or the antisense CAG-encoding polyglutamine peptide serves as the pathogenic moiety in Huntington's disease like-2 (HDL2). In this issue of *Neuron*, Wilburn et al. show that in a HDL2 mouse model, the polyglutamine peptide drives disease progression.

Among the first microsatellite expansion diseases identified 20 years ago was the X-linked, CAG trinucleotide repeat disorder spinobulbar muscular atrophy (SBMA, or Kennedy's disease) (La Spada et al., 1991). In SBMA and eight additional neurodegenerative diseases, the CAG repeat is located within the open reading frame and encodes a stretch of glutamines (Orr and Zoghbi, 2007), providing the basis for their designation as polyglutamine (polyQ) expansion disorders. The most recent polyQ expansion disease identified, SCA17, came to light 10 years ago (Nakamura et al., 2001). However, over the last decade, no additional neurodegenerative syndromes have qualified as polyQ expansion diseases, although others have been suggested as candidates. Two in particular, SCA8 and Huntington's disease like-2 (HDL2), map to loci containing an unstable CAG repeat. SCA8 is a slowly progressive neurodegenerative disease arising from a CTG/CAG expansion located on chromosome 13q21 (Koob et al., 1999), while HDL2 is associated with a CTG/CAG repeat at the *Junctophilin-3* (*JPH3*) locus with the CTG repeat on the *JPH3* sense strand (Holmes et al., 2001). It is important to note that while the molecular mechanism or mechanisms underlying the polyQ diseases are a matter of considerable investigation and discussion, a basic tenant of the field is that the polyQ-containing protein/peptide is the pathogenic entity. However, to date there is biochemical evidence only for the CUG-repeat-containing transcript, and not the polyQ-encoding transcript, in SCA8 and HDL2 in humans (Koob et al., 1999; Holmes et al., 2001). Furthermore, the CUG-containing RNA species can be as toxic as the polyQ peptide, as exemplified in the myotonic dystrophies DM1 and DM2 (Ranum and Cooper, 2006). As such, the focus has been on whether the CUG-containing strand, which encodes a detectable RNA in SCA8 and HDL2, is the pathogenic culprit. In fact, for both SCA8 and HDL2, there is evidence to suggest involvement of a toxic RNA species in disease progression (Daughters et al., 2009; Rudnicki et al., 2007). Yet, particularly for HDL2, there remains a puzzling feature of its pathology, where like those of HD, HDL2 brains contain intranuclear inclusions that react with the polyQ antibody 1C2 (Rudnicki et al., 2008). The presence of polyQ-1C2-positive inclusions suggests that two mechanisms might contribute to disease; a toxic -RNA encoded by one strand and a polyQ peptide encoded by the other (Figure 1).

In an attempt to clarify the molecular source of pathogenicity in HDL2, Yang and colleagues (as detailed in this issue of *Neuron*) engineered a series of transgenic mice expressing human *JPH3* mutant alleles. Using bacterial artificial chromosomes (BACs), an approached pioneered by the Yang group for the study of HD, Wilburn et al. (2011) generated transgenic mice carrying 165 kb from the *JPH3* locus with ~120 CAG repeats. These mutant *JPH3* BAC mice presented with several key features found in HDL2 patients. Among these are an age-dependent motor deficit, forebrain atrophy, and the presence of nuclear inclusions positive for ubiquitin and reactive with two polyQ antibodies, including 1C2. At a molecular level, Yang and colleagues provide evidence for a novel promoter that drives expression of a polyQ-encoding transcript from the DNA strand in the antisense orientation to *JPH3*. Importantly, Wilburn et al. provide biochemical evidence that mutant BAC-JPH3 brains express insoluble polyQ peptides of a size range that would be expected to be encoded by the *JPH3* CAG antisense strand of the BAC transgene. However, the nuclear inclusions in the mutant BAC-JPH3 mice are also positive for RNA from the sense CUG strand. Given this finding, which of the two transcripts is the pathogenic species in the BAC-JPH3 mice? Does disease progression require functional expression of both transcripts?

To address the extent to which the CAG-polyQ-encoding antisense transcript contributes to pathogenesis, Wilburn et al. developed a second version of an expanded CAG repeat BAC-JPH3 transgenic mouse, designated BAC-HDL2-STOP. In this mouse, exon 1 of the *JPH3* transgene was replaced with a previously well-characterized transcription STOP sequence such that expression of the *JPH3* CUG sense strand is selectively silenced while expression of the antisense CAG transcript remains intact. By behavioral and neurological measures, the BAC-HDL2-STOP mice expressing only the CAG antisense transcript develop motor deficits and degenerative pathology very similar to that seen in the original BAC-JPH3 mice expressing both transcripts. Although the extent to which the JPH3 sense CUG transcript contributes to disease was not assessed directly, these results provide strong evidence that the CAG antisense transcript is very pathogenic and a prominent contributor to disease progression in this mouse model of HDL2. In a final series of studies, Wilburn

et al. provide evidence that, like other polyQ disorders (e.g., HD, SCA3, and SBMA), the nuclear inclusions in mutant BAC-JPH3 mice and HDL2 patients are positive for the CREB binding protein (CBP) and that CBP-mediated transcription is disrupted, extending the pathogenic similarities between HD and the BAC-JPH3 mice by identifying a shared pathogenic mechanism of transcriptional dysfunction.

In sum, Wilburn et al. present compelling evidence that the phenotype of their BAC-JPH3 mice meets the two major criteria for classification as a polyQ-based neurodegenerative disease. Mutant BAC-JPH3 mice express a biochemically detectable polyQ peptide that is sufficient to cause disease. Since pathogenicity of the CUG-containing strand in absence of a CAG transcript was not examined, these studies do not rule out the possibility that, in part, a toxic RNA from the sense CUG strand may contribute to disease. However, given the robust disease phenotype in the BAC-HDL2-STOP mice, its seems very likely that if a CUG sense RNA contributes to disease in this model, it does so to a far lesser extent than the antisense-encoded polyQ peptide.

Perhaps the more gripping question is whether the work of Wilburn et al. is sufficient to justify admission of HDL2 to the group of human polyQ expansion neurodegenerative diseases. Without question, the work of Wilburn et al. demonstrates a very elegant murine genetic approach for ascertaining the biological impact of an antisense CAG transcript and provides support for HDL2 being a polyQ disease. Yet one absolutely crucial piece of data remains elusive. On the one hand, Wilburn et al. illustrate the many pathological similarities between HDL2 and the polyQ disease HD, including the presence of polyQ-1C2-positive nuclear inclusions in the brains of HDL2 patients. However, unlike as in HD, there is no direct evidence in humans to suggest that the *JPH3* antisense CAG transcript is a stable RNA transcript that encodes a polyQ peptide. Wilburn et al. suggest several possible reasons for the inability to detect either the *JPH3* antisense CAG transcript or the polyQ protein. For example, they point out that the inability to detect either the *JPH3* antisense CAG transcript or the polyQ protein in HDL2 patient brains might reflect the loss of neurons expressing them in the disease, a feature not seen in the BAC-JPH3 mice. Nevertheless, the fact remains that in HD patient brains, a *HD* CAG transcript and huntingtin protein are readily detectable. If HDL2 indeed shares a polyQ pathogenic mechanism with HD, why has it been difficult to provide molecular evidence for *JPH3* CAG/polyQ expression in humans? Given the findings presented by Wilburn et al., it is worth pursuing RNA sequencing studies in human patient populations to provide direct evidence of the wild-type JPH3 antisense CAG transcript. In the absence of such data, one needs to keep in mind that the BAC-JPH3 model of HDL2 was generated using a repeat size considerably longer than that seen in HDL2 patients (120 Qs versus 50 Qs, respectively). Accordingly, it seems prudent to recognize this caveat when considering the relevance of the mouse model to the human disease. Nonetheless, the work by Yang and colleagues paves the way for future studies aimed at extending the polyQ-mediated mechanism of pathology to humans with HDL2.

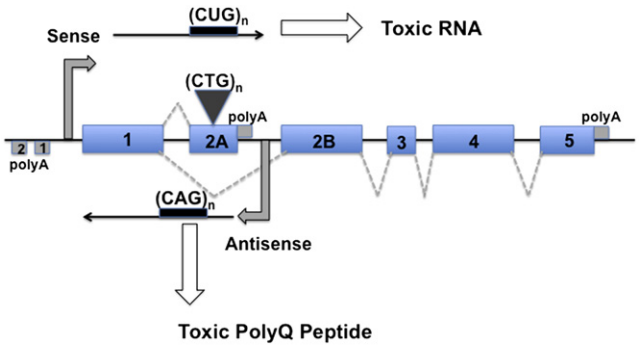

Figure 1. The *JPH3* Locus Indicating Bidirectional Transcription that Generates a Sense CUG Transcript and an Antisense polyQ-Encoding CAG Transcript

The blocky gray arrows indicate transcription start sites. *JPH3* exons and introns are depicted with the CTG/CAG repeat located in alternatively spliced exon 2a. Small gray boxes depict polyA addition sites. The transcriptional start site for the CAG (antisense *JPH3*) transcript is positioned based on the results of Wilburn et al. (2011).

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