**Abstract**

Introduction: Frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) are devastating neurodegenerative disorders with genetic, neuropathological, and clinical overlap. A hexanucleotide (GGGGCC) repeat expansion in C9ORF72 is the major genetic cause of both diseases. It is currently unknown whether the C9ORF72 repeat expansion causes FTD/ALS or is not disease causing or merely a genetic marker. We hypothesized that sense and antisense transcripts of the expanded GGGGCC repeat form nuclear RNA foci and also undergo repeat-associated non-ATG (RAN) translation resulting in the production of aggregation-prone proteins. The goals of this study are to examine whether antisense transcripts resulting from bidirectional transcription of the expanded repeat behave in a similar manner, and to examine the relationship between foci formation and RAN translation.

Methods: To examine RAN translation from sense and antisense transcripts of the expanded C9ORF72 repeat, we generated novel rabbit polyclonal antibodies for the 5 potential c9RAN proteins: poly(GA), poly(GR), poly(GP), poly(PA), and poly(PR). These antibodies were used to analyze the presence of c9RAN proteins in brain tissue from FTD/ALS cases with or without the expanded C9ORF72 repeat. To investigate foci formation from sense and antisense transcripts, RNA fluorescence was carried out on spinal cord, frontal cortex, and cerebellum sections of FTD/ALS cases using probes to sense (GGGGCC) or antisense (CCCCCGG) repeats. To examine the relationship between foci and RAN translation, tissue sections subjected to FISH were subsequently stained using a poly(GP) antibody followed by a fluorescent-labeled secondary antibody.

Results: Foci composed of sense or antisense transcripts are observed in the frontal cortex, spinal cord and cerebellum of FTD/ALS cases, and neuronal inclusions of poly(GP), poly(PA) and poly(PR) are present in various brain tissues in FTD/ALS, but not in other neurodegenerative diseases, including CA1 repeat disorders. Although RNA foci and poly(GP) inclusions infrequently co-occur in the same cell, the brain region sampled (frontal cortex vs. cerebellum) and foci type (antisense vs. sense) both significantly affect the percentage of cells having both foci and inclusions, despite similar frequency of sense and antisense inclusions.

Discussion: That foci and poly(GP) inclusions are seldom observed in the same cells suggests that one event may preclude the other, and that they represent two distinct ways in which the C9ORF72 repeat expansion may evoke neurotoxic effects.

**Conclusion**

Through the production of sense and antisense repeat RNA and five c9RAN proteins, the C9ORF72 repeat expansion leads to the production of seven potentially toxic biomolecules. It is now of importance to determine if and how these biomolecules contribute to FTD/ALS pathogenesis, and whether the frequency or regional localization of RNA foci and c9RAN inclusions correlate with distinct clinical features.

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