Isoform-level analyses of ALS and related disorders using innovative long-read sequencing technologies

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Aberrant RNA splicing is a significant contributor to the pathogenesis of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD), with TDP-43 mislocalization and aggregation representing a central pathological hallmark. However, the mechanisms driving these changes are not fully understood. While short-read RNA sequencing has uncovered gene-level changes and disrupted pathways, it lacks the resolution to fully characterize splicing abnormalities. Hence, we are using long-read sequencing technologies to accurately capture the transcript diversity and alternative splicing. Long-read RNA sequencing has been employed to analyze post-mortem motor cortex tissue from ~100 ALS/FTLD-spectrum patients (with and without C9orf72 repeat expansions) and ~20 control individuals using the Kinnex method (Revio, PacBio). A custom pipeline incorporating tools like Iso-Seq, FLAIR, is used to classify transcripts as well as quantify gene- and isoform-level expression. Multiple transcripts obtained are classified into major structural categories, including full splice matches, incomplete splice matches, and novel transcripts. Genes and individual transcripts are then quantified, enabling the identification of isoform-specific differences in expression and detection of alternative splicing events, thereby nominating disease-relevant genes, transcripts, and/or pathways. Complementary short-read RNA-seq data from the same samples is integrated to enhance quantification and statistical power. Our integrative approach provides a detailed view of transcriptomic alterations in ALS and its spectrum of disorders, highlighting the potential of long-read technologies to reveal novel disease mechanisms. These insights may contribute to improved molecular classification, diagnostics, and therapeutic target discovery in ALS and related neurodegenerative disorders.

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