Expression profiling of FTD patients with mutations in MAPT, GRN and c9orf72

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Research question and background

To better understand the molecular mechanisms underlying the very distinct genetic, pathological and clinical subtypes of frontotemporal dementia (FTD) we performed genome-wide cap-analysis gene expression (CAGE) coupled to next-generation sequencing on RNA from seven different brain regions from patients with GRN, MAPT, c9orf72 mutations and controls. The ultimate goal of our research is to create cellular models that truly mimic disease and that can be used to find ideal targets for future therapy.

Methods and tissues used

Frozen brain material was collected from seven neurologically normal aged individuals and five FTD patients from each of the following groups: GRN mutation carriers, P301L MAPT mutation carriers, and c9orf72 hexanucleotide expansion carriers. Total RNA was extracted from seven brain regions: caudate, putamen, hippocampus, frontal, temporal and occipital lobes and cerebellum using the Trizol® method followed by purification with Rneasy columns. CAGE libraries containing 27-nt-long tags corresponding to the initial bases at the 5′ ends of capped RNAs were prepared for each sample as previously described (1). Single read CAGE libraries were then sequenced with an Illumina Hiseq sequencer.

Results and conclusion

Genome-wide expression analysis CAGE and next-generation sequencing has been performed to detect genes and gene-specific networks that are disrupted by mutations in MAPT, GRN and c9orf72.

Because of the nature of the technique utilized for these experiments, expression in both the qualitative and quantitative levels has been detected for all 5'-capped RNAs including unknown transcripts and non-coding RNAs like antisense transcripts. Although the analysis is still ongoing preliminary results show that the majority of differentially expressed genes between cases and controls are present in the frontal and temporal regions. Of the differentially expressed genes more than 50% are up-regulated and involved in extracellular matrix and cell matrix interactions while the down regulated genes are mainly involved in synaptic functions.

Among the different mutation carriers the c9orf72 expansion patients present less differentially expressed genes as compared to controls.

Common pathways show to be involved in all the FTD subtypes including the protein A signaling pathway, RAR activation pathway and Wnt-β catenin pathway.

We have also identified several long noncoding RNAs (lincRNAs) differentially expressed between cases and controls. In particular some lincRNAs up regulated in MAPT mutation carriers are predicted to bind to proteins splicing factors and preliminary studies after knocking them down in neuroblastoma show an effect on total or t 3R/4R tau ratio highlighting their potential roles in the disease pathogenesis.

References:

1) Takahashi H, Lassmann T, Murata M**,** Carninci P. 5'end-centered expression profiling using cap-analysis gene expression and next generation-sequencing. Nat Protoc. 2012 23;7(3):542-61