Institute of Psychiatry, Psychology & Neuroscience

Neuroscience MSc

Differential Expression of Transposable Elements in Amyotrophic Lateral Sclerosis

William Lizr, Esteban Gomez Cifuentes, Alfredo Iacoangeli

KING'S College LONDON

Background:

Previously unexamined regions of the genome have the potential to explain the missing heritability of ALS. Transposable elements (TEs) make up about 40% of the human genome but have not been included in whole-genome transcriptomics studies because they contain highly repetitive elements which are difficult to sequence.

ALS risk mutations can be categorized into 2 types:

a) DNA damage and repair.

b) Genes associated with long-axon metabolism of motor neurons.

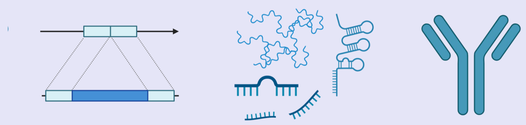
ALS pathology is consistent with the hypothesis that TEs are inserting into open chromatin (highly transcribed genes in motor neurons) and damaging them, thus triggering a DNA repair response.

Transposons are active within the genome in 3 ways:

i. Autonomous transposons like *LINE, Alu, SVA* transpose into new genomic loci generating **Retrotransposon Insertion Polymorphisms (RIPs).**

ii. Non-autonomous transposons produce regulatory **non-coding RNAs.**

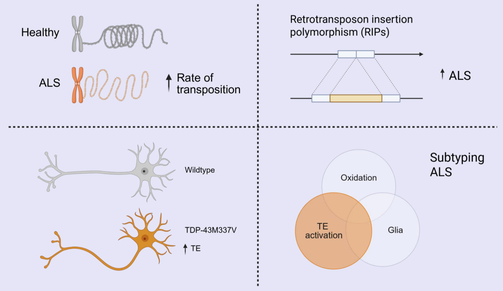
iii. Human endogenous retroviruses participate in immune response to viral infection via **signaling protein** encoded in their envelope gene.



Note. Representative figures (left to right): RIPs, ncRNAs, ERV env protein

Converging evidence suggests TE expression is increased in ALS:

1. ALS patients have **abnormal chromatin conformation** of specific retrotransposon loci leading to increased transposition activity (Savage et al., 2020)
2. ALS patients have increased numbers of Retrotransposon Insertion Polymorphisms (RIPs) leading to DNA damage. (Savage et al., 2022)
3. Mutant induced motor neurons (TDP-43M337V) with a TDP-43 mutation develop increased levels of TEs because they are unable to effectively repress them. (Valdebenito-Maturana et al., 2022)
4. A subset of ALS patients have globally upregulated expression of transposable elements leading to overactivation of TE-repressing mechanisms. (Tam et al., 2019)



Note. Left to right: (Top) De-repression of TEs via chromatin conformation; RIPs (bottom) induced motor neuron model of TDP pathology; subtypes of ALS pathology

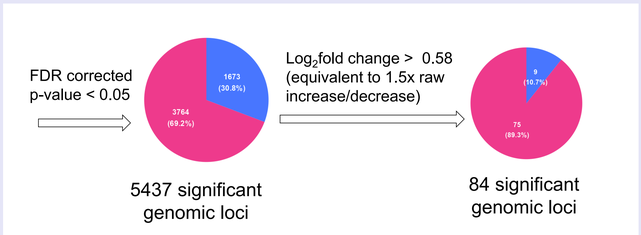
Aims:

1. Investigate whether **transposon expression is increased** in primary motor cortex neurons of ALS patients.
2. Investigate which specific **transposon classes, families and loci show increased or decreased transcription**

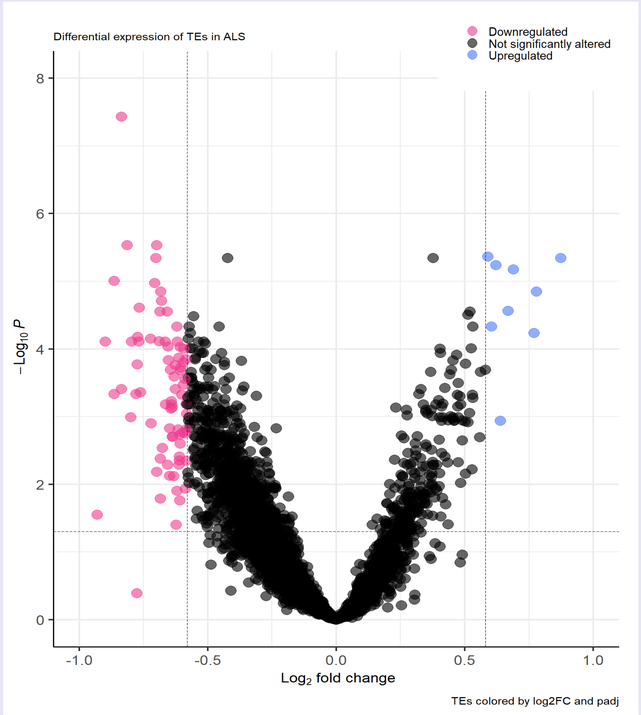
Methods:

- Differential expression analysis of TEs was performed using DESeq2. An existing RNA-seq dataset was obtained from KCL brain bank which consisted of RT-PCR measurements of transcripts from **primary motor cortex tissue** taken from **frozen postmortem samples of ALS patients** and healthy controls.
- Transposon expression was quantified using **SQuiRE** by distributing multi-mapped reads based on the existing distribution of unique reads.
- False discovery rate correction was applied using the **Benjamini–Hochberg (BH) procedure**.

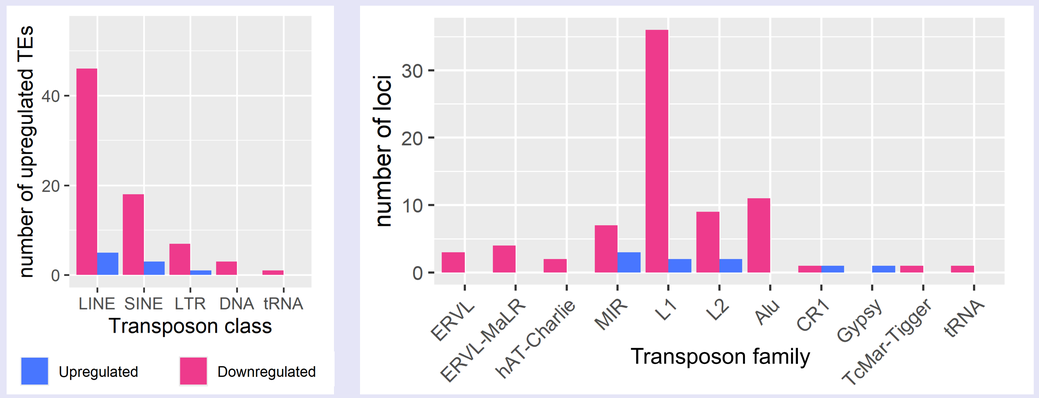
Results:



Note. (Above) Number of significantly differentially expressed TE loci before and after filtering for 1.5x increase/decrease



Note. Volcano plot of differential expression. X-axis represents effect size of increase/decrease. Y-axis represents multiple comparisons-adjusted p-value



Note. (Left panel) Count of upregulated and downregulated TE loci belonging to each transposon class; (Right panel) Count of upregulated and downregulated loci belonging to each TE family.

Conclusions:

- **The vast majority TE loci are downregulated, however 10 – 30% of TE loci are upregulated.**
- **Some loci of Mammalian interspersed repeats (MIRs) and L2 LINEs are slightly upregulated. These two types classes of TEs interact to alter expression patterns by affecting broad genomic regulatory regions. Both elements being upregulated suggests large-scale transcriptional changes in ALS patients.**
- Canonically active Alu elements are not significantly upregulated. LINE-1 elements make up the largest proportion of transposons with significantly increased transcription rates above levels of biological significance (> 1.5x increase).
- Mammalian endogenous retroviruses (MIRs) are activated in ALS suggesting a low-level immune signaling response.

Future directions:

1. Combining **genomics** and **chromatin sequencing:** To verify the assertion that RIPs are causing DNA damage in heavily transcribed regions of ALS motor neurons, genomics must be combined with chromatin conformation measurements to ascertain whether open chromatin regions in ALS pathology contain more non-reference RIPs.
2. Combining **transcriptomics** and **genomics**: Rates of RIP generation need to be corroborated with actual measurements of non-reference RIPs in ALS patients' genomes to determine whether abnormal placement of RIPs is associated with progression and symptoms.
3. Methods of aligning genomic fragments must progress to adjust for disruptions of reference genome coordinates. Without de novo genome assembly of ALS genomes, RIPs will be discarded as low-quality reads.

References: Savage, A. L., Iacoangeli, A., Schumann, G. G., Rubio-Roldan, A., Garcia-Perez, J. L., Al Khleifat, A., Koks, S., Bubb, V. J., Al-Chalabi, A., & Quinn, J. P. (2022). Characterisation of retrotransposon insertion polymorphisms in whole genome sequencing data from individuals with amyotrophic lateral sclerosis. *Gene*, *843*, 146799. https://doi.org/10.1016/j.gene.2022.146799; Savage, A. L., Lopez, A. I., Iacoangeli, A., Bubb, V. J., Smith, B., Troakes, C., Alahmady, N., Koks, S., Schumann, G. G., Al-Chalabi, A., & Quinn, J. P. (2020). Frequency and methylation status of selected retrotransposition competent L1 loci in amyotrophic lateral sclerosis. *Molecular Brain*, *13*(1), 154. https://doi.org/10.1186/s13041-020-00694-2; Tam, O. H., Rozhkov, N. V., Shaw, R., Kim, D., Hubbard, I., Fennessey, S., Propp, N., Phatnani, H., Kwan, J., Sareen, D., Broach, J. R., Simmons, Z., Arcila-Londono, X., Lee, E. B., Deerlin, V. M. V., Shneider, N. A., Fraenkel, E., Ostrow, L. W., Baas, F., … Hammell, M. G. (2019). Postmortem Cortex Samples Identify Distinct Molecular Subtypes of ALS: Retrotransposon Activation, Oxidative Stress, and Activated Glia. *Cell Reports*, *29*(5), 1164-1177.e5. https://doi.org/10.1016/j.celrep.2019.09.066; Valdebenito-Maturana, B., Rojas-Tapia, M. I., Carrasco, M., & Tapia, J. C. (2022). Dysregulated Expression of Transposable Elements in TDP-43M337V Human Motor Neurons That Recapitulate Amyotrophic Lateral Sclerosis In Vitro. *International Journal of Molecular Sciences*, *23*(24), 16222. https://doi.org/10.3390/ijms232416222