



University
of Exeter

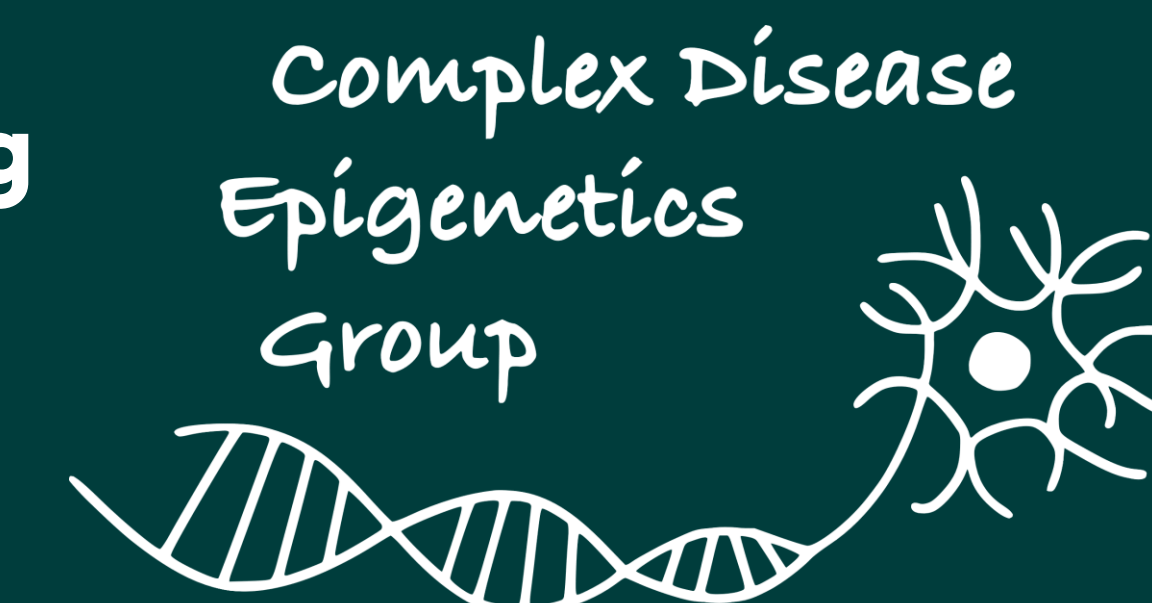
Quantifying full-length circular RNAs in Alzheimer's disease using cutting edge long-read sequencing

Morteza Kouhsar^{1,*}, Adam R Smith¹, Ehsan Pishva², Katie Lunnon^{1,*}

1. Department of Clinical and Biomedical Sciences, Faculty of Health and Life Sciences, University of Exeter, Exeter, United Kingdom.

2. Department of Psychiatry and Neuropsychology, School for Mental Health and Neuroscience (MHeNs), Faculty of Health, Medicine and Life Sciences (FHML), Maastricht University, Maastricht, The Netherlands.

* Corresponding authors



Background

The amount of protein that is made in a cell is regulated by the amount of its gene that is expressed (the messenger RNA-mRNA). There are many ways that cells regulate mRNA expression, and a growing area of interest is in circular RNAs (circRNAs), which are circular RNA structures that are complimentary to the mRNA and can affect its translation into a protein. Circular RNAs (circRNAs) are stable, covalently closed RNA molecules crucial for gene regulation, notably by acting as microRNA sponges [1-3]. Emerging evidence suggests that their dysregulation contributes to Alzheimer's disease (AD) pathogenesis by influencing amyloid-beta production, neuronal function, neuroinflammation, and oxidative stress [4-8]. However, quantifying circRNAs is challenging due to sequence homology with their linear mRNA cognates. Long-read sequencing platforms, like Oxford Nanopore Technologies (ONT), offer an accurate solution for their full-length characterization [9-12]. This study aims to use these advanced techniques to identify and quantify full-length circRNAs that are dysregulated in the prefrontal cortex of AD patients. Using long-read sequencing will generate a high-resolution dataset of full-length circRNA sequences, improving our understanding of their structure and function in AD. The research will also explore interactions between circRNAs, microRNAs and mRNAs, potentially revealing new disease mechanisms.

Methods

Post-mortem brain tissue was obtained from 12 AD samples and 12 age and sex-matched non-demented elderly controls. Total RNA was extracted, followed by ribosomal RNA (rRNA) depletion. To enrich for circRNAs, samples were treated with RNase R to degrade linear RNA. The enriched RNA was then reverse-transcribed into cDNA, poly(A)-tailed, and converted to double-stranded cDNA. cDNA quality and concentration were assessed using an Agilent TapeStation. Libraries were prepared using the ONT ligation sequencing kit and sequenced on a PromethION flow cell. Raw sequencing data was basecalled, and the resulting reads were mapped to the human reference genome (GRCh38). Full-length circRNAs were then quantified using the CIRI-long software [10, 12], and a differential gene expression analysis was performed using the limma package in R [13].

Results

Nanopore sequencing identified numerous circRNAs differentially expressed between AD and control brain samples. By integrating these findings with existing miRNA and mRNA data, we are identifying key regulatory networks and novel gene targets involved in AD pathology. This multi-omics approach highlights specific circRNA-miRNA-mRNA axes that may drive disease mechanisms.

Conclusions

This study demonstrates that RNase R treatment and Oxford Nanopore sequencing is an effective method for profiling full-length circRNA expression in human brain tissue. The circRNA isoforms identified provide a valuable resource for investigating their function in Alzheimer's disease.

Funder

NIHR | Exeter Biomedical
Research Centre

Contact details:

Web: <https://www.epigenomicslab.com/>
Email: m.kouhsar@exeter.ac.uk

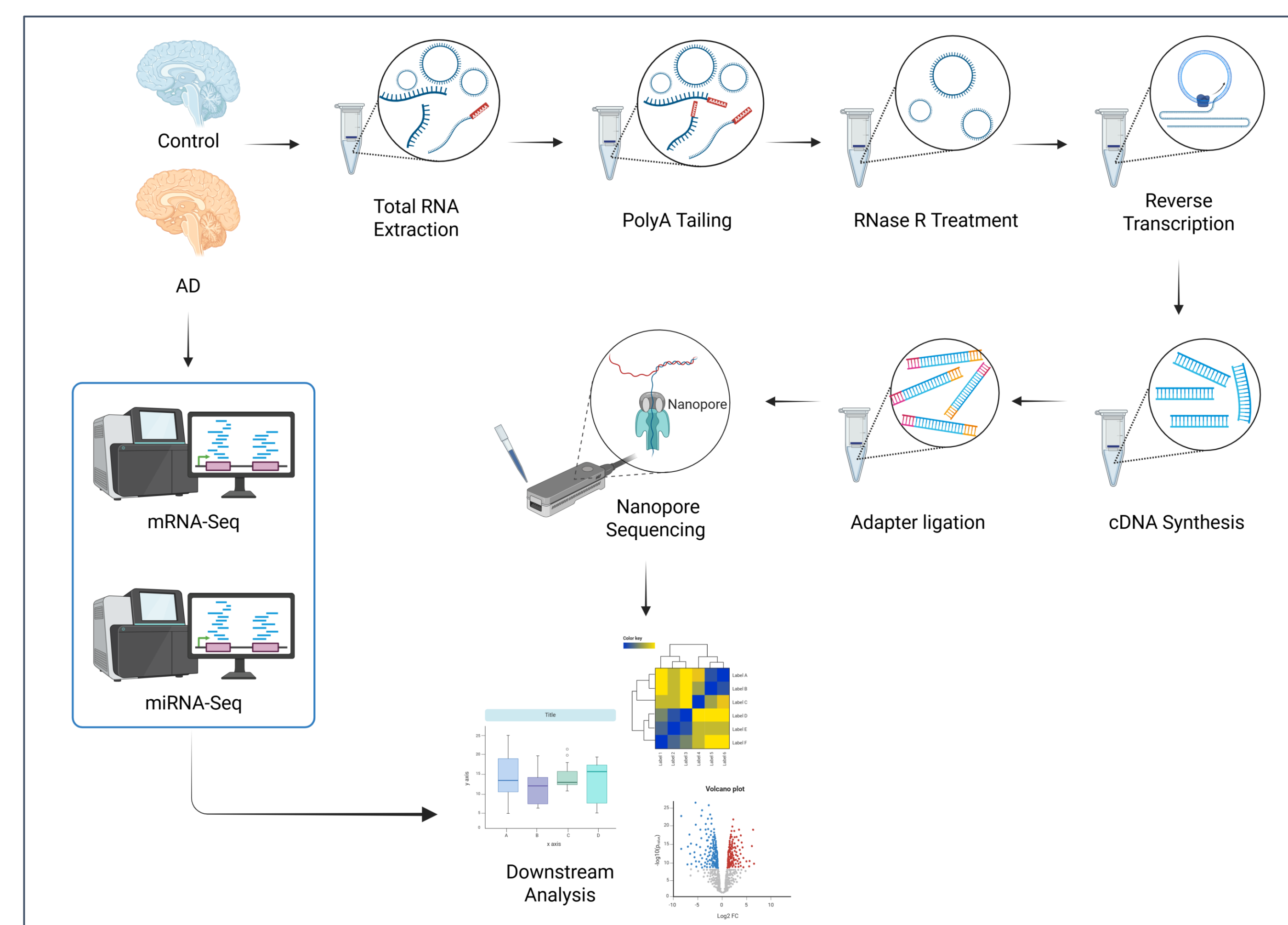


Fig1. The overall workflow of the study.

References

- Kristensen, L.S., et al., The biogenesis, biology and characterization of circular RNAs. *Nat Rev Genet*, 2019. 20(11): p. 675-691.
- Li, X., L. Yang, and L.L. Chen, The Biogenesis, Functions, and Challenges of Circular RNAs. *Mol Cell*, 2018. 71(3): p. 428-442.
- O'Leary, E., et al., The therapeutic potential of circular RNAs. *Nat Rev Genet*, 2025. 26(4): p. 230-244.
- Beylerli, O., et al., CircRNAs in Alzheimer's disease: What are the prospects? *Noncoding RNA Res*, 2024. 9(1): p. 203-210.
- Dube, U., et al., An atlas of cortical circular RNA expression in Alzheimer disease brains demonstrates clinical and pathological associations. *Nat Neurosci*, 2019. 22(11): p. 1903-1912.
- Puri, S., et al., Identification of circRNAs linked to Alzheimer's disease and related dementias. *Alzheimers Dement*, 2023. 19(8): p. 3389-3405.
- Mo, D., et al., Circular RNA Encoded Amyloid Beta peptides-A Novel Putative Player in Alzheimer's Disease. *Cells*, 2020. 9(10).
- Lu, Y., L. Tan, and X. Wang, Circular HDAC9/microRNA-138/Sirtuin-1 Pathway Mediates Synaptic and Amyloid Precursor Protein Processing Deficits in Alzheimer's Disease. *Neurosci Bull*, 2019. 35(5): p. 877-888.
- Xin, R., et al., isoCirc catalogs full-length circular RNA isoforms in human transcriptomes. *Nat Commun*, 2021. 12(1): p. 266.
- Zhang, J., et al., Comprehensive profiling of circular RNAs with nanopore sequencing and CIRI-long. *Nat Biotechnol*, 2021. 39(7): p. 836-845.
- Liu, Z., et al., circFL-seq reveals full-length circular RNAs with rolling circular reverse transcription and nanopore sequencing. *Elife*, 2021. 10.
- Hou, L., J. Zhang, and F. Zhao, Full-length circular RNA profiling by nanopore sequencing with CIRI-long. *Nat Protoc*, 2023. 18(6): p. 1795-1813.
- Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK, limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research*, 2015. 43(7): e47.