Research proposal

Does RNA-editing change Alzheimer’s disease

# Summary

The purpose of this study is to look at the role of RNA-editing alterations in Alzheimer's disease (AD) and how they could affect the creation of biomarkers and therapy strategies. Alzheimer's disease (AD) is a neurological condition marked by cognitive decline, memory loss, and aberrant behaviour. Aberrant RNA editing has been linked to a number of disorders, including Alzheimer's disease (AD), where dysregulated editing affect gene expression and protein function, aiding in the development of the disease. A quantitative strategy will be used to accomplish these research goals, concentrating on locating RNA-editing sites and evaluating the quantities of RNA editing in AD and control samples. There will be 16 volunteers total in the trial, including 8 AD patients and 8 controls. The study intends to assess if there are substantial variations in RNA-editing patterns between the two groups through thorough analysis. The study plan will include details on the precise procedures employed for the examination of RNA-editing. This work seeks to advance our comprehension of this complicated neurodegenerative disorder by investigating the molecular pathways driving AD and finding possible RNA-editing alterations related to the disease. The discovery of certain RNA-editing alterations might also result in the creation of fresh biomarkers for the diagnosis and prognosis of AD. The new summary highlights the significance of researching aberrant RNA editing in AD, whereas the prior summary omitted crucial background information on RNA editing and its possible consequences for disease. Therefore, in order to gain important insights into the role of RNA editing in AD pathogenesis and open up new diagnostic and therapeutic possibilities, the emphasis is on using a quantitative method to identify RNA-editing sites and measure editing levels.

# Background to the Study

Alzheimer's disease (AD) is a fatal neurological condition marked by deteriorating mental abilities, memory loss, and behavioural abnormalities. The most common kind of dementia, Alzheimer's disease (AD), affects millions of people globally. There are around 50 million AD sufferers worldwide, therefore, AD is the primary cause of dementia globally, accounting for 60–70 per cent of all cases of dementia (Wu et al., 2023). Other kinds of dementia exist, such as vascular dementia and Lewy body dementia, but AD stands out owing to its high frequency and the significant effects it has on cognitive function, memory, and everyday functioning. Moreover, planning for public health, providing care, and creating effective interventions to address this serious health issue all depend on knowing how common AD is. Despite intensive research, the particular molecular pathways behind AD development still need to be better known. RNA editing has recently been identified as a possible regulatory mechanism involved in the onset and development of several neurological diseases, including AD (Gardner et al., 2019). The post-transcriptional modification procedure known as RNA editing modifies the nucleotide sequence of RNA molecules (Costa Cruz and Kawahara, 2021). Adenosine-to-inosine (A-to-I) editing, the most common kind of RNA editing in mammals, is mediated by enzymes called adenosine deaminases acting on RNA (ADAR).

Adenosine is changed into inosine by deamination by ADAR enzymes, especially ADAR1 and ADAR2. These enzymes identify double-stranded RNA structures (Salvetat et al., 2022). During translation, inosine is converted to guanosine, possibly changing the sequence of a protein such as the A-to-I editing of glutamate receptor subunits in the brain. Long double-stranded RNA structures like inverted repeats or duplexes created by base-pairing between exonic and intronic sections are the main locations where A-to-I RNA editing occurs (Konen et al., 2020). A growing body of research indicates that the deregulation of RNA-editing mechanisms contributes to AD aetiology (Wu et al., 2023). There is a link between RNA editing and AD, as several studies have found abnormal RNA-editing patterns in AD patients compared to healthy people. Xu et al. (2017) found elevated amounts of A-to-I RNA editing in a number of AD-related genes, including presenilin 1 (PSEN1), apolipoprotein E (APOE), and amyloid precursor protein (APP), in postmortem brain samples from patients with Alzheimer's disease (AD). The analysis showed that, in comparison to control samples, there were higher amounts of editing taking place at particular places within these genes. The APP produces the amyloid-beta (A) peptides that cause the recognisable plaques seen in AD brains. While APOE is a significant genetic risk factor for late-onset AD, PSEN1 is a part of the -secretase complex that produces A (Tassinari et al., 2023). Changes in RNA-editing activity within these genes have an impact on how they are expressed, how proteins work, and how diseases develop as a result.

Additionally, Wu et al. (2023) discovered that the 5-HT2C serotonin receptor had changed editing levels, which is connected to the onset of AD and cognitive decline. A possible role in the pathophysiology of AD and cognitive impairment was suggested by the study's findings on changes in the amounts of RNA editing that take place at particular locations within the 5-HT2C receptor gene. Neuronal activity and cognition have been connected to serotonin signalling, and disease related to AD has been linked to dysregulation of the 5-HT2C receptor. In Alzheimer's disease (AD), changes to the 5-HT2C receptor's RNA editing have an impact on the receptor's functionality and cause problems with serotonin signalling. This change might be a factor in the deterioration of cognitive function seen in AD patients. RNA-editing dysregulation in AD have important functional ramifications (Tarozzi et al., 2022). RNA-editing activities can affect RNA secondary structure, RNA-protein interactions, and alternative splicing, among other RNA biology characteristics. These alterations eventually impact protein synthesis, stability, location, and function, which contribute to neurodegeneration in AD. Altered RNA-editing levels result in the creation of structurally and functionally abnormal protein isoforms (Costa Cruz and Kawahara, 2021). For instance, A-to-I editing in the glutamate receptor subunit GluA2's coding sequence might cause an arginine codon (AGA) to be changed into an early stop codon (IGA), resulting in a shortened and nonfunctional protein. The availability of binding sites for RNA-binding proteins, the stability of RNA, and the regulation of alternative splicing are all potential effects of RNA editing (Moore et al., 2019). Disruptions in these systems hasten the start and course of AD and have cascading effects on cellular homeostasis, protein synthesis, and gene expression. It is crucial to appreciate the specific RNA-editing modifications that take place in AD and their functional implications in order to properly comprehend the disease processes and identify potential therapy targets (Wu et al., 2023). Furthermore, examining how RNA editing is involved in Alzheimer's disease (AD), researchers can pinpoint specific abnormalities in RNA-editing patterns that are connected to the condition. These findings shed light on the underlying molecular mechanisms driving the evolution of AD and propose fresh therapeutic targets for specialist therapy approaches. Moreover, finding different RNA-editing patterns also help in the creation of novel biomarkers that will aid in the precise diagnosis and prognosis of AD, enabling early identification and individualised treatment plans. For instance, particular RNA-editing sites linked to Alzheimer's disease (AD) or tailored modulation of ADAR enzymes is used to cure the condition (Salvetat et al., 2022). It could be able to limit the course of the disease and cognitive decline seen in AD by altering the RNA-editing processes at these precise sites, which would also fix aberrant protein sequences and restore normal editing patterns.

Recent research has shed further light on the probable processes driving the dysregulation of RNA editing in AD. Variations in the expression and activity of ADAR enzymes impact the RNA-editing modifications connected to AD (Gardner et al., 2019). The brain is the main spot of expression for ADAR1 and ADAR2, which perform different but related RNA-editing tasks. Alterations in the expression levels or decreased enzymatic activity of ADAR enzymes cause abnormal RNA-editing patterns in AD. Alterations to the ADAR enzymes are not the exclusive cause of RNA-editing dysregulation in AD (Salvetat et al., 2022). For instance, modifications to the RNA-binding proteins (RBPs) that interact with AD AR enzymes and control their activity have an impact on the amounts of RNA editing. RBPs perform crucial functions in RNA metabolism, including RNA-editing activities, by binding to specific RNA sequences and impacting RNA structure and function (Dick et al., 2019). Disruptions in the interaction between RBPs and ADAR enzymes, which affect their recruitment to certain RNA targets, the cause of dysregulated RNA-editing events in AD. The dysregulation of RNA editing in AD also attributed to epigenetic changes such as DNA methylation and histone modifications, according to current study. Epigenetic modifications have an effect on the production and activity of ADAR enzymes as well as the accessibility of RNA substrates for editing (Konen et al., 2020). For instance, variations in the levels of ADAR expression in gene regulatory areas may affect the results of RNA editing. Furthermore, modifications to chromatin's accessibility and structure have an impact on how readily available double-stranded RNA substrates for ADAR enzymes are. Therefore, it is essential to understand the functional implications of this dysregulation in order to comprehend the role of RNA-editing dysregulation in the aetiology of AD (Tassinari et al., 2023). Modified RNA-editing events can have an influence on a number of biological systems, including neurotransmission, synaptic plasticity, and neuroinflammation, which are known to be impacted in AD. For instance, changes in the amounts of RNA editing in genes that are involved in synaptic function and plasticity, such as glutamate receptors, ion channels, and scaffolding proteins, have an impact on neuronal excitability and synaptic transmission and result in cognitive impairment in AD.

Additionally, RNA editing disruption impact neuroinflammatory and immunological responses in AD. ADAR enzymes have been shown to regulate the expression of immune-related genes by editing the RNA transcripts of these genes, such as those encoding innate immune system components and pro-inflammatory cytokines (Welden et al., 2022). The synthesis and function of these immune components can be affected by the ADAR enzymes through RNA editing, which have an impact on the immune response and inflammation seen in Alzheimer's disease (AD). This demonstrates the therapeutic potential of focused modulation of RNA-editing mechanisms to modify immunological dysregulation and reduce the neuroinflammatory component of AD. Chronic neuroinflammation and abnormal immunological responses, two hallmarks of AD, result from dysregulated RNA-editing in the disease. Different experimental approaches can be used to examine AD's RNA-editing changes. By locating RNA-editing sites and calculating the extent of their editing, transcriptomic studies, such as RNA sequencing, can give extensive information regarding RNA-editing profiles (Gardner et al., 2022). Bioinformatics methods and algorithms created particularly for detecting RNA-editing events, such as A-to-I RNA-editing, can be used to find differentially edited sites in AD compared to healthy controls. Candidate locations for RNA editing will be found by bioinformatic research and comparison with reference genomes. Quantitative methods, such as the application of software tools made expressly for RNA editing analysis, will be used to determine the extent of editing taking place at these locations. The quantitative analysis will provide the frequency or fraction of modified reads at each candidate site, revealing the degree of RNA editing in AD. The work intends to identify RNA-editing alterations and their possible functional significance in AD pathogenesis by fusing RNA-seq data with computational analysis (Wu et al., 2021).

# Aims

This study aims to determine the role of RNA editing in Alzheimer's disease (AD) and its potential implications for the pathogenesis of the disease.

Specifically, the research objectives are as follows:

* To investigate factors influencing RNA-editing dysregulation in AD.
* To identify perceived functional consequences of altered RNA-editing in AD.
* To uncover potential therapeutic implications of targeting RNA-editing in AD.

# Methodology

## Research Method

This study will use interpretivism as its research technique, which will provide a complete understanding of the function of RNA editing in Alzheimer's disease (AD) and its implications for the aetiology of the illness. A crucial aspect of the interpretivist strategy is comprehending people's arbitrary impressions and interpretations of RNA-editing modifications in AD (Ma et al., 2021). By starting with specific observations and going on to more broad-based hypothese or generalisations, this study use an inductive research approach to investigate qualitative data in order to produce new insights and ideas (Broesch et al., 2020). This strategy is especially helpful for studying unique and complex processes, such as RNA-editing modifications in AD, since it promotes the development of inventive hypotheses and new findings (Sandberg et al., 2022).

## Research Design

It is critical to understand that a qualitative research technique will be employed to examine how individuals perceive and interpret RNA-editing changes in AD in order to acquire a complete understanding of the phenomena being examined (Sandberg and Rossi, 2022). Data gathered in a number of methods using the flexibility of qualitative research technique, including document analysis, interviews, observations, and participant observation. The primary method of data collection for this study will be interviews, which will allow an in-depth analysis of participants' perspectives, areas of expertise, and thoughts on RNA-editing dysregulation in AD (Broesch et al., 2020). The use of interviews encourages the collection of detailed and rich data through open-ended questions and questioning.

## Selection of Participants

Moreover, to choose participants for the study, a purposive selection approach called snowball sampling will be used. The first participants will be found through current networks, and after that, they will be requested to suggest any further possible participants they believe would be qualified (Shay, 2021). This sampling technique will make it easier to identify people who have particular knowledge or experience in RNA editing and AD, producing a sample that is varied and information-rich.

## Data Analysis

The qualitative information gathered from the interviews will be examined using thematic analysis. In order to comprehend the participants' viewpoints on RNA-editing dysregulation in AD, this method entails finding common themes, patterns, and categories within the data (Sandberg et al., 2022). The data will be coded, categorised, and interpreted as part of the iterative analysis process in order to identify key themes and provide a thorough grasp of the research issue.

# Ethical Declaration & Research Integrity

This study project complies with the ethical guidelines established by the institutional review board in order to preserve participant rights, privacy, and informed consent (Ienca and Ignatiadis, 2020). All volunteers will be asked for their informed permission after being fully informed of the study's objectives, methods, potential risks, and rewards. At any time, participants will be allowed to leave the study without suffering any repercussions. Personal identifying information will be anonymised or pseudonymized throughout the study to preserve participant confidentiality and anonymity (Zhang et al., 2019). During data collection, analysis, and reporting, each participant will be given a special identification or pseudonym in place of their real names. This will assist protect their privacy and stop their identify from being revealed (Okpala and Korzeniowska, 2023). Field notes, interview transcripts, and digital files—all of the study's data—will be safely preserved in accordance with institutional guidelines and privacy regulations. Only authorised members of the study team will be able to access the data since it will be kept on password-protected storage devices (Sim and Waterfield, 2019). Hard copies of the material will be maintained in a closed, secure location with only authorised researchers being given limited access. The research team is dedicated to preserving the highest standards of ethical behaviour and openness in the scientific community (De Bie et al., 2023). Data shall be gathered, examined, and interpreted impartially and objectively. The integrity of the study findings shall be ensured by acknowledging and addressing any conflicts of interest or possible biases. The study will be rigorously carried out, using the proper research procedures, and in accordance with ethical standards and scientific principles. The results of this study will be published in credible, peer-reviewed journals with an emphasis on neurology, neurobiology, or Alzheimer's research (Naik et al., 2022). Therefore, to connect with the scientific community and advance knowledge in the subject, journals will be chosen based on their reputation, relevance to the research topic, and impact factor.

# Milestones of the Study

Moreover, to assure the progression and completion of the study project, the study will follow a sequence of milestones. The many actions engaged in the study must be organised and managed according to these milestones. A thorough literature review, planning the research methods, conducting interviews to gather data, analysing the data collected, submitting draft work to the supervisor for feedback, holding meetings to discuss the draft and make necessary revisions, finalising the project's conclusions, and finishing the final draft are among the milestones. These checkpoints are planned out over several months, enabling a methodical and well-organised evolution of the study.

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| **Date** | **Activities** |
| 29/05/23 | Background reading of the research project |
| 05/06/23 | Proposal/initial meetings with the supervisor |
| 19/06/23 | Literature review |
| 25/06/23 | Research Methods planning |
| 27/07/23 | Collection of Data |
| 18/07/23 | Analysis of the Data collected |
| 26/07/23 | Submit some draft work to the supervisor |
| 30/08/23 | Second meeting with the supervisor to discuss the draft |
| 05/08/23 | Further draft |
| 10/08/23 | Discussion on conclusions of the project with a supervisor |
| 16/08/23 | Further drafts |
| 20/08/23 | Final meetings with the supervisor |
| 25/08/23 | The final draft of the project |

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# Interview Questions

1. Can you share your understanding of RNA editing and its potential role in Alzheimer's disease?
2. What do you believe are the key factors contributing to RNA-editing dysregulation in AD?
3. In your opinion, how might altered RNA editing impact the function of genes and proteins relevant to AD pathology?
4. Can you describe any specific examples or cases where RNA-editing changes have been observed in AD?
5. From your perspective, how important is it to investigate the functional consequences of altered RNA editing in AD?
6. What are your thoughts on the potential therapeutic implications of targeting RNA-editing processes in the context of AD treatment?
7. Are there specific RNA-editing sites or genes that you believe hold particular significance in AD pathogenesis? If so, please elaborate.
8. How do you think RNA-editing alterations interact with other molecular processes in AD, such as neuroinflammation or protein aggregation?
9. From your knowledge or expertise, what challenges or limitations do you foresee in targeting RNA-editing for therapeutic interventions in AD?
10. In your view, what potential future directions or areas of research should be explored to advance our understanding of RNA-editing dysregulation in AD?