Comparative Analysis of MAPT-RNA Expression Levels Across Temporal and Visual Cortices

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Abstract

Tau aggregation and dysregulation have been associated with various neurodegenerative disorders, such as Alzheimer's Disease and various types of frontotemporal dementia. These disorders, known as taupathies, can present clinically as behavioral disorders, movement disorders, language disorders, and dementia. The heterogeneity of clinical presentation among taupathies and the lack of reliable biomarkers for early diagnosis hinders the ability to diagnose taupathies before they have reached stages at which they are mostly untreatable. For most of these disorders, the primary brain region of hyperphosphorylated, or mutant tau accumulation, has not been identified. Higher expression of MAPT-RNA is more likely to lead to higher deposition and potentially accumulation of mutant tau; therefore, identifying regions with high-tau expression can be a valuable approach to identifying potential sites of tau accumulation. Though significant heterogeneity in regions of interest (ROI) of MAPT-RNA expression and tau accumulation has been found, investigating potential ROIs that can be generalized to small populations or even individuals can serve as a diagnostic tool in taupathies. By identifying a primary region of accumulation, check-ups and early diagnosis may be made available to those affected by risk factors for taupathies. Therefore, this study will analyze the expression locations of the human MAPT gene RNA in post-mortem brain samples from two healthy controls to determine if a cortical region with specifically high MAPT-RNA expression can be identified and suggested as a potential ROI for tau accumulation.
Keywords: MAPT, tau, frontotemporal dementia, MAPT-RNA expression, tauopathy, tau accumulation

1. Introduction

1.1 The Human MAPT Gene

The human MAPT gene, which encodes for tau protein, is located on chromosome 17q21.31. The mRNA transcripts of the MAPT gene undergo alternative splicing in exons 2, 3, and 10, leading to 6 tau isoforms in the central nervous system (Hernandez et al., 2024).. There have been links found between more than 50 MAPT mutations and various neurodegenerative disorders such as frontotemporal dementia with Parkinsonism-17 (FTDP17), corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), Pick's disease, and Alzheimer's disease (AD). Understanding the expression of the MAPT gene is important for understanding the regulation of tau protein levels, which can lead to neurodegenerative disorders if found in excess.

1.2 Tau Proteins

Tau proteins are a part of the microtubule-associated protein (MAP) family. In humans, tau proteins are most commonly present in neuronal cells, though smaller amounts are also found in peripheral tissues such as the heart, kidney, and lung. In the neuron, tau is abundant in the axons, though it is also present in smaller quantities in dendrites. Under physiological conditions, the tau protein's primary functions are stabilizing neuronal microtubules, facilitating axonal transportation, and modulating synaptic plasticity (Buchholz & Zempel, 2024). There are 6 brain-specific isoforms of the tau protein. These isoforms are differentiated by the number of amino-terminal inserts (0N, 1N, or 2N) or carboxyl-terminal repeat domains (3R or 4R). The loss of function, mislocalization, or toxic gain of function of pathological tau may lead to neurotoxicity and also mediate amyloid-β-induced toxicity (Wang & Mandelkow, 2016). As tau is a natively unfolded protein that doesn't tend to aggregate, mutations or post-translational modifications may lead to tau aggregation and tau-induced neurodegeneration. Therefore, it is important to identify brain regions in which more tau proteins are synthesized, as those regions have a higher probability of having tau pathology by simple probability. The present study evaluates MAPT-RNA expression to identify predicted regions of high tau deposition.

1.4 Location of Tau aggregates

Raised phosphorylated tau levels in the cerebrospinal fluid are a biomarker for neurodegenerative diseases such as Alzheimer's disease. CSF p-tau181, p-tau217, and p-tau231 have been shown to be accurate predictors of cognitive impairment in AD patients (Ashton et al., 2022). However, obtaining CSF tau measurements requires an invasive spinal tap procedure and does not provide significant information on the spatial distribution of tau (Dani et al., 2016). Moreover, potential treatments for diagnoses provided by spinal taps are limited when spatial location of tau dysfunction is not known. Though it is commonly held that tau-pathology progression follows a distinct pattern, identified post-mortem through approximating Barack Stages, recent studies have concurred that this homogenous approach is limited and that individual differences lead to different progression patterns (St-Onge et al., 2023). Therefore, it is more effective to track pathological tau accumulation and spread by using individualized regions of interest (Sohn et al., 2015). In this study, we examine differences in MAPT gene expression in post-mortem brain samples from two healthy control subjects to evaluate expression differences between the temporal and visual cortices. The object of this study is to determine if a significant difference in MAPT-RNA expression can be identified based on the cortex.

2. Procedure

2.1 Subjects

Samples were obtained from the Allen Institute's 1,000 Gene Survey in Cortex as part of the Allen Human Brain Atlas. The Allen Institute obtained frozen tissue samples from adult male and female control or schizophrenia subjects from the brain tissue collection of the Section on Neuropathology, Clinical Disorders Branch, GCAP, IRP, National Institute of Mental Health, NIH, Bethesda, MD, and the University of Miami Brain Endowment Bank, University of Miami Miller School of Medicine, Miami, FL. All control subjects had normal neuropathological examination

results, no known history of neuropsychiatric disease, and no evidence for substance use. The male and female control subjects from whose samples were tagged for MAPT-RNA were used in this study. Both of the subjects were 41 years old (Technical White Paper: In Situ Hybridization in the Allen Human Brain Atlas, 2013).

2.2 Tissue Samples

Frozen tissue samples, sectioned into coronal slabs of 1-1.5 cm thickness, were harvested by the Allen Institute. The samples from the male subject used in this study were taken from the right hemisphere, and the samples from the female subject were taken from the left hemisphere. For the male subject, 4 slices from the temporal cortex and 4 slices from the visual cortex were analyzed; for the female subject, 3 slices from the temporal cortex and 3 slices from the visual cortex were analyzed. Visual cortex samples included Brodmann's areas 17 and 18, and temporal cortex samples included Brodmann's areas 21 and 22 (Technical White Paper: In Situ Hybridization in the Allen Human Brain Atlas, 2013).

2.3 Image Analysis

ISH expression images obtained from the Allen Institute's Allen Brain Atlas data portal were manually cropped on ImageJ/FIJI (NIH). Copies of the cropped images were separately thresholded for high and moderate expression levels. The cells appearing red in the original images exhibit high MAPT-RNA expression, the cells that appear green exhibit moderate MAPT-RNA expression, and the cells that appear blue exhibit low MAPT-RNA expression per ISH guidelines. Accepted RGB color model hues for high-expression (red) were 1-23, and moderate-expression (green) were 53-123. Low-expression (blue) cells were discarded as the blue hues were too similar to the background and could result in misleading data. The circularity parameters used to extract the data were set at 0.70-1.00. Percent areas, the total area of cells within the accepted hues divided by the total area of the cropped image, were calculated for each thresholded crop using FIJI.

Differences in percent areas were analyzed across different cortices and sexes for both expression levels. Statistics were run as two-way ANOVA tests on GraphPad Prism 10. The significance level was 0.05.

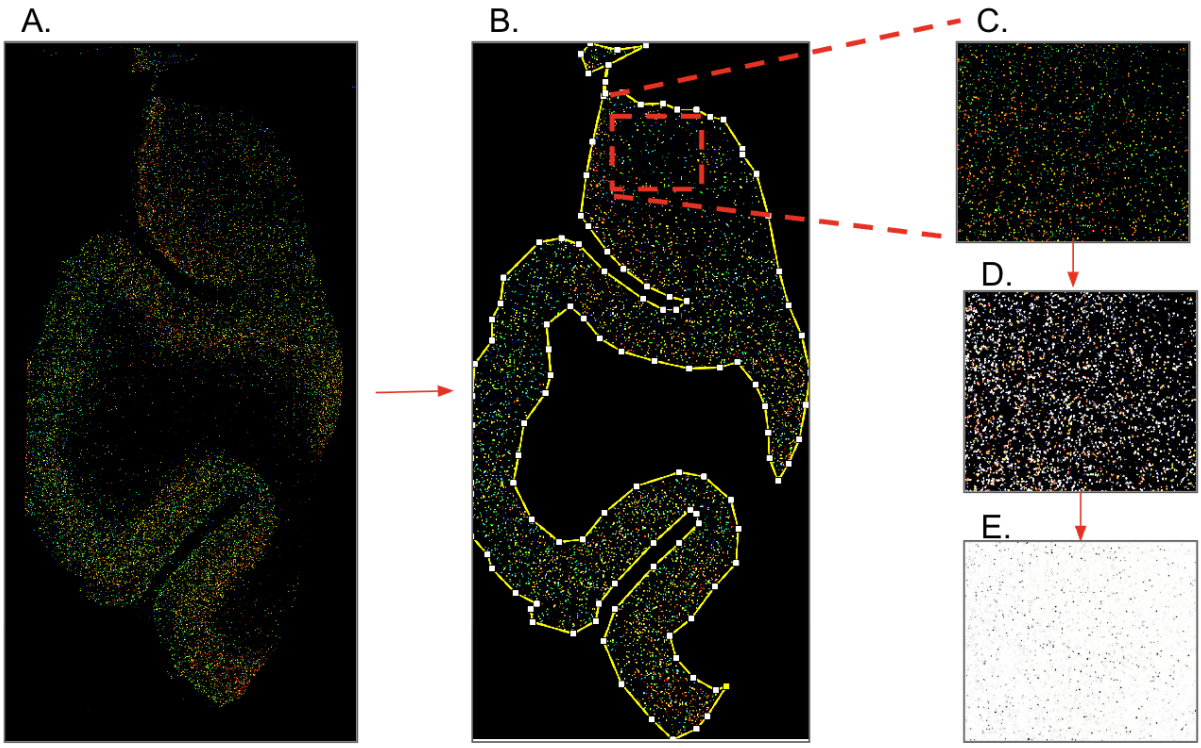

Figure 1. Image Analysis Pipeline

(A) Example ISH expression image data obtained from the Allen Institute's Allen Brain Atlas data portal.
(B) Manual cropping of the ISH expression image.
(C) Example insert to demonstrate marker expression at low, medium, and high levels.
(D) The sample was thresholded to only include the cells with the accepted green hues.
(E) Masks were obtained from the analysis of accepted cells and used for percent area calculations on FIJI.

Data Organization, Analysis, and Visualization

The extracted datawere organized Google Sheets based on subject and brain region to identify differences based on these factors. T-tests and 2-way ANOVAs were then run on GraphPad Prism 10 (San Diego, CA) for all comparisonswith an alpha level of 0.05. The 2-way ANOVA test included a column effect, row effect and interaction effect. The difference between visual and temporal cortex analyses or both subjects and the difference between the subjects for both brain regions was calculated. Tukey's correction was used for multiple comparisons. The resulting graphs from the 2-way ANOVA test were also generated on Prism 10.

Results

Figure 2 shows the percent area for high-level expression (red) cells in the samples from the visual and temporal cortices of both the male and female subjects. A 2-way ANOVA revealed a significant difference in high-level MAPT-RNA expression between the visual and temporal cortex in the male subject ($F_{1,10} = 16.37$, p=0.002). However, no significant difference was observed between the visual and temporal cortices in the female subject (p=0.891). There was no significant difference based on subject or sex-related differences in the expression of

MAPT-RNA in the visual ($F_{1,10} = 0.8$, p=0.156) or the temporal cortex ($F_{1,10} = 0.8$, p=0.296). Post-hoc comparisons demonstrate that the male high-level MAPT expression is higher in the visual cortex compared to the temporal (**, p<0.01).

Figure 3 shows the percent areas for medium-level expression (green) cells in the samples from the visual and temporal cortices of both the male and female subjects. A 2-way ANOVA revealed a significant difference in medium-level MAPT-RNA expression between the visual and temporal cortex in the male subject (p<0.001). However, no significant difference was observed between the visual and temporal cortices in the female subject (p>0.999) ($F_{1,10} = 0.9$, p=0.345). The 2-way ANOVA also revealed a significant difference in medium-level MAPT-RNA expression in the temporal cortex between the male and female subjects (p=0.018) ($F_{1,10} = 18.0$, p=0.002). No significant difference was observed in the visual cortex between the male and female subjects (p=0.165). Post-hoc comparisons demonstrate that the male medium-level MAPT expression is higher in the visual cortex compared to the temporal cortex, and that the medium-level MAPT expression is higher for the female subject compared to the male subject in the temporal cortex. (**, p<0.01).

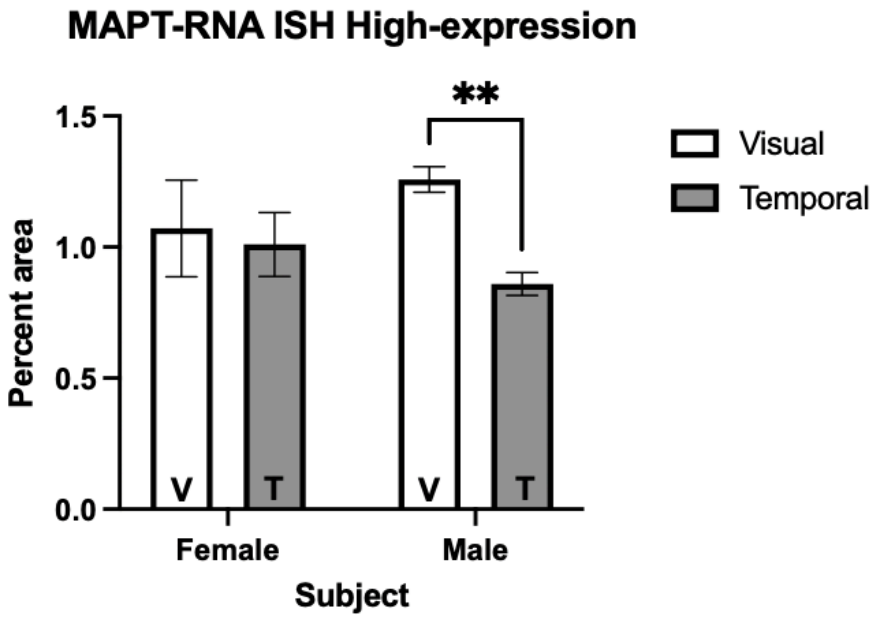

Figure 2. A 2-way ANOVA revealed a main effect of brain region. Post-hoc comparisons revealed a significant difference between the visual and temporal cortices in the male subject (p=0.002, **).

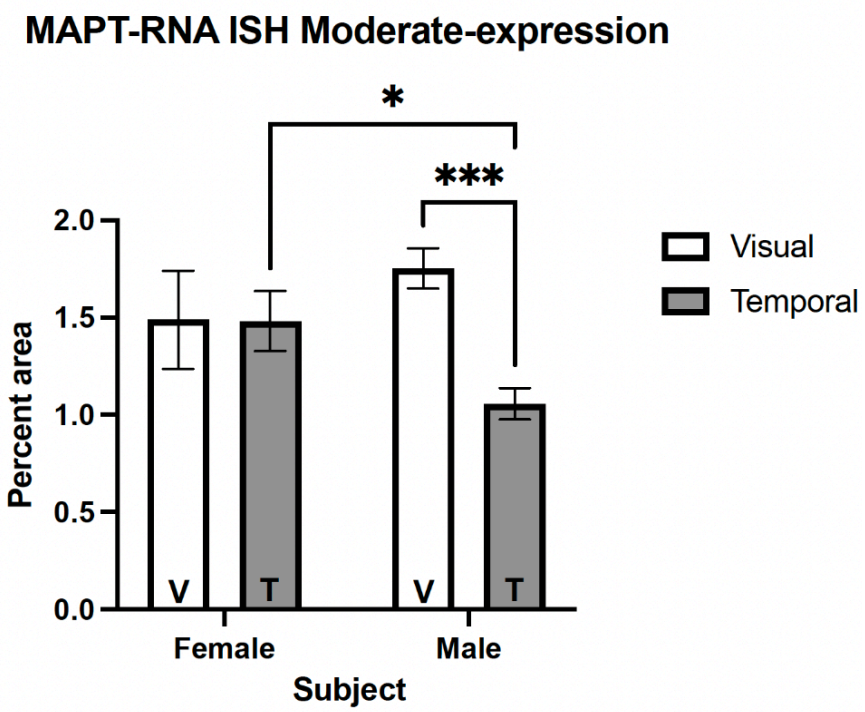

Figure 3. A 2-way ANOVA revealed a main effect of brain region and subject. Post-hoc comparisons revealed a significant difference between the visual and temporal cortices in the male subject, and between the male and female subject in the temporal cortex ($p<0.001$, ***) ($p=0.018$, *).

Discussion

The analysis of the results shows a significant difference in high MAPT-RNA expression between the visual and temporal cortices of the male subject, and a significant difference in moderate MAPT-RNA expression between the temporal cortices of the male and female subjects and between the visual and temporal cortices of the male subject. There is no trend in results that could be observed in both subjects in either high or moderate expression. This might be due to the low sample size or a broader lack of homogeneity in MAPT-RNA expression among people. Previous studies have also highlighted the heterogeneity among individuals regarding regions of tau accumulation (St-Onge et al., 2023). The differences observed within moderate MAPT expression show that the same heterogeneity can also be seen in MAPT gene expression. Though the main demographical factor separating the subjects was their sex, this finding is more likely caused by individual differences rather than a difference between sexes in general. Similarly, the results are unlikely to be related to the difference in the hemisphere between the subjects, as the sample size is not high enough for a generalization, and there have been no previous reports of the left or right hemisphere being more susceptible to tau aggregation or MAPT expression. Thus, more extensive studies are needed to understand the potential inter-region, inter-sex, and inter-individual differences of MAPT expression in humans, its implications for susceptibility to tau dysfunction.

A previous study by Lee and colleagues has identified the entorhinal cortex (A28/34, A35/36r) as a region with high tau-PET signaling and the first region to become tau positive, specifically in AD patients (Lee et al., 2022). Their study has also identified the inferior temporal gyri as a “propagation hub”, a brain region well-positioned to promote widespread tau aggregation (Lee et al., 2022). In light of such findings, our study examined MAPT expression in the temporal and visual cortices of healthy controls. The study aimed to compare subjects to understand if a generalization on a brain region in which MAPT expression was more concentrated can be made. A specific region that had significantly increased MAPT expression would be more likely to have higher tau expression and to be a potential “propagation hub”. If such a region exists, its identification can be a useful starting point for developing potential novel diagnostic methods and treatments for taupathies. In our analysis, MAPT-RNA expression was found to be more in the visual cortex, particularly for the male subject. However, the sample size for this study is inadequate to make such a generalization on a population level. Moreover, these results also do not indicate that high MAPT-RNA expression can be linked to susceptibility to tau aggregation, as only healthy controls were included in the study.

Post-translational modifications are the most prominent cause of tau aggregation (Hattiholi et al., 2025). Specifically, mutations that alter phosphorylation sites of tau may lead to hyperphosphorylation. Hyperphosphorylation of tau is three times higher in the disease state, compared to physiological conditions (Nisbet et al., 2015). Hyperphosphorylation of tau compromises microtubule stabilization and axonal transport by negatively regulating the binding of tau to microtubules. Hyperphosphorylation significantly reduces tau’s affinity for microtubules (Geschwind, 2003). It has been hypothesized that reducing MAPT mRNA expression and thus tau protein levels can prevent tau aggregation and could be a potential treatment option for tauopathies (Sud et al., 2014). Both Sud and colleagues and Chakravarthy and colleagues have been successful in developing antisense oligonucleotides against MAPT, which have reduced the expression of MAPT mRNA and tau levels. MAPT-RNA expression can offer valuable insight into tau accumulation. Identifying general brain regions with high MAPT-RNA expression can help us identify regions that are potentially more susceptible to tau accumulation and can be promising sites for further research on the diagnosis and treatment of tauopathies.

Conclusion

The findings of this study confirm that there are significant differences between different ROIs in individuals and also between individuals concerning MAPT-RNA expression. These differences suggest that it is difficult to determine potential tau accumulation sites that can be generalized to all individuals. However, identifying ROIs that are more susceptible to tau aggregation can be a useful diagnostic tool for smaller populations or at the individual level. More extensive future studies that have higher sample sizes and that examine a wider range of brain regions are required to identify specific trends for populations.

Acknowledgments

I would like to thank [name redacted by Managing Editor], for his guidance and mentorship in developing this project, training me on the methodologies, and for reviewing and editing the paper.

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EDITOR COMMENTS AND RECOMMENDATION:
The reviewers congratulate the author for tackling such an interesting and complicated topic. While both reviewers find the submission promising, they agree that major revisions are necessary prior to publication. The editor advises the student to pay particular attention to the reviewer suggestions regarding improvement of clarity and organization of the manuscript, including a more thorough discussion of rationale. Given the reviewer feedback, the editor's recommendation is to review the manuscript with major revisions.

REVIEWER 1:

This manuscript addresses an important question about MAPT-RNA expression and its potential role in tauopathies. The research problem is relevant, and the use of atlas data shows initiative. The main concern is writing clarity and organization. Many sentences are long, repetitive, or overly technical, making the argument difficult to follow. The introduction provides useful background but should be restructured, incorporate more explanation of referenced material and revised to highlight the research gap more clearly. The discussion is too lengthy and repeats earlier material; it should be refocused on key findings, limitations, and future directions. In contrast, the conclusion is a very short summary- this may just be reorganized.

Additional notes: references need consistent formatting, some terms should be more clearly defined for the journal's audience, and the abstract should be shortened to emphasize the study's aims and results.

Overall, the paper has promise but requires major revisions to improve clarity, concision, and alignment of sections.

Overall Recommendation: Major revisions required.

REVIEWER 2:

Overall Summary

The topic is interesting and relevant to understanding regional vulnerability to tau pathology, and the paper demonstrates engagement with the literature on tau biology. However, there are several concerns that need to be addressed. The paper would benefit from a more clearly framed rationale for comparing temporal versus visual cortices, additional information on methods/statistical analyses, and consistent reporting of statistical results. Importantly, more

caution should be taken for overstating conclusions based on this analysis as the statistical approach may not be appropriate for such a small sample.

Major Comments:

- Introduction
  - The introduction should have more citations throughout
  - "The object of this study is to determine if a significant difference in MAPT-RNA expression can be identified based on the cortex." - This part of the paper frames the objective of your study and the research question. Thus it should more clearly lay out:
  - Why have you chosen to compare temporal and visual cortices?
  - What would a significant difference between these cortices in MAPT-RNA expression point to?
  - Given your analysis is of health controls, how does this information inform us on tauopathies?
  - The research question is phrased as looking for whether there is a "significant difference" – which could sound mechanical and is better phrased scientifically
  - Lay out the rationale more clearly for why a difference in the MAPT-RNA expression of these cortices at baseline (in healthy individuals) should theoretically indicate which regions are more vulnerable to tau pathology and thus potential sites of early identification in tauopathies

- Methods:
  - Why were those hue/circularity thresholds chosen? Cite previous literature or explain how this was chosen.
  - Explicitly state what the independent and dependent variables are in the 2 way ANOVA - not just "column" and "row"
  - The text does acknowledge this but statistical testing (2-way ANOVA and post-hoc Tukey) is not meaningful with n=1 per group. These tests assume multiple independent samples per condition, not multiple slices from the same individual. Thus, the paper should clarify that the analyses reflect within-subject variability across slices, not population-level inference and that statistical tests here are descriptive.
  - There is some redundancy in the last two sections of the Methods. This could be condensed into one section or perhaps make one into a Statistical Analysis section.

- Results:
  - The reporting of 2-Way ANOVA is confusing: "A 2-way ANOVA revealed a significant difference in high-level MAPT-RNA expression between the visual and temporal cortex in the male subject (16.37, p=0.002). However, no significant difference

was observed between the visual and temporal cortices in the female subject (p=0.891).” – a 2-way ANOVA cannot tell the difference is within the male as it analyzes across both. Did you mean that there was a significant difference between cortices (across both subjects) but that this was stronger in males in post-hoc tests? Or the interaction of sex and cortex was significant?

- Make sure reporting of statistics is consistent: ensure each result is accompanied by F/T-statistics and p-value, and that the test you used is explicitly stated. Also, some stats have ** and some do not.

- Discussion
  - “Though the main demographical factor separating the subjects was their sex, this finding is more likely caused by individual differences rather than a difference between sexes in general.” – The current analysis cannot differentiate these two sources of variation. I recommend to either cite previous work showing no sex differences or phrase it is not possible to determine if this is sex or individual differences.
  - “Their study has also identified the inferior temporal gyri as a “propagation hub”, a brain region well-positioned to promote widespread tau aggregation (Lee et al., 2022). In light of such findings, our study examined MAPT expression in the temporal and visual cortices of healthy controls.” – This should be mentioned earlier in the paper. Did this lead you to have a hypothesis that the temporal regions would have more MAPT-RNA compared to visual? If so, this should be made clear in the intro and you should discuss how you found the opposite and consider why.
  - The phrasing implies too much causality at times. For example: “A specific region that had significantly increased MAPT expression would be more likely to have higher tau expression and to be a potential “propagation hub”. MAPT-RNA expression does not equal tau aggregation and so this should be more cautiously phrased.
  - “Post-translational modifications are the most prominent cause of tau aggregation (Hattiholi et al., 2025). Specifically, mutations that alter phosphorylation sites of tau may lead to hyperphosphorylation. Hyperphosphorylation of tau is three times higher in the disease state, compared to physiological conditions (Nisbet et al., 2015).
    Hyperphosphorylation of tau compromises microtubule stabilization and axonal transport by negatively regulating the binding of tau to microtubules. Hyperphosphorylation significantly reduces tau’s affinity for microtubules (Geschwind, 2003).” – This section would be more appropriate in the introduction

Minor comments:

- “Taupathies” should be “tauopathies” throughout the paper

- Abstract: “Higher expression of MAPT-RNA is more likely to lead to higher deposition and potentially accumulation of mutant tau; therefore, identifying regions with high-tau expression can be a valuable approach to identifying potential sites of tau accumulation.” This sentence is unclear - do you mean to say mapping regions with high levels of MAPT-RNA could help identify potential sites of tau accumulation.

- Abstract: “Though significant heterogeneity in regions of interest (ROI) of MAPT-RNA expression and tau accumulation has been found, investigating potential ROIs that can be generalized to small populations or even individuals can serve as a diagnostic tool in taupathies.” Meaning of this sentence is unclear.
- Abstract” Therefore, this study will analyze the expression locations of the human MAPT gene RNA in post-mortem brain samples from two healthy controls to determine if a cortical region with specifically high MAPT-RNA expression can be identified and suggested as a potential ROI for tau accumulation.” This should be in past tense and might be good to frame it as a small exploratory analysis to avoid overstating the generalizability
- Introduction: Section 1.3 is missing
- Introduction: “There have been links found between more than 50 MAPT mutations and various neurodegenerative disorders such as frontotemporal dementia with Parkinsonism-17 (FTDP17), corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), Pick’s disease, and Alzheimer’s disease (AD).” This sentence has no citation. It may also be helpful to provide information about what changes to tau biology are associated with some of these mutations

Comparative Analysis of MAPT-RNA Expression Levels Across Temporal and Visual Cortices

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Abstract

Tau aggregation and dysregulation have been associated with various neurodegenerative disorders, such as Alzheimer's Disease and various types of frontotemporal dementia. The heterogeneity of clinical presentation among tauopathies and the lack of reliable biomarkers for early diagnosis hinder the ability to diagnose tauopathies before they have reached stages at which they are mostly untreatable. For most of these disorders, the primary brain region of hyperphosphorylated, or mutant tau accumulation, has not been identified. Higher expression of MAPT-RNA is more likely to lead to higher deposition and potentially accumulation of mutant tau; therefore, mapping regions with high levels of MAPT-RNA could help identify potential sites of tau accumulation. Although significant heterogeneity in regions of interest (ROI) of MAPT-RNA expression and tau accumulation has been found, identifying potential ROIs that can be generalized to small populations can be valuable in diagnosis and treatment. This study is a small-scale exploratory analysis of the expression locations of the human MAPT gene RNA in post-mortem brain samples from two healthy controls, aimed at seeing if the temporal or visual cortex is comparatively more susceptible to tau accumulation.
Keywords: MAPT, tau, frontotemporal dementia, MAPT-RNA expression, tauopathy, tau accumulation

1. Introduction

1.1 The Human MAPT Gene

The human MAPT gene, which encodes for tau protein, is located on chromosome 17q21.31. The mRNA transcripts of the MAPT gene undergo alternative splicing in exons 2, 3, and 10, leading to 6 tau isoforms in the central nervous system (Buchholz & Zempel, 2024). There have been links found between more than 50 MAPT mutations and various neurodegenerative disorders such as frontotemporal dementia with Parkinsonism-17 (FTDP17), corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), Pick's disease, and Alzheimer's disease (AD) (Strang et al., 2019). Understanding the expression of the MAPT gene is important for understanding the regulation of tau protein levels, which can lead to neurodegenerative disorders if found in excess.

1.2 Tau Proteins

Tau proteins are a part of the microtubule-associated protein (MAP) family. In humans, tau proteins are most commonly present in neuronal cells, though smaller amounts are also found in peripheral tissues such as the heart, kidney, and lung (Xu et al., 2025). In the neuron, tau is abundant in the axons, though it is also present in smaller quantities in dendrites. Under physiological conditions, the tau protein's primary functions are stabilizing neuronal

microtubules, facilitating axonal transportation, and modulating synaptic plasticity (Buchholz & Zempel, 2024). There are 6 brain-specific isoforms of the tau protein. These isoforms are differentiated by the number of amino-terminal inserts (0N, 1N, or 2N) or carboxyl-terminal repeat domains (3R or 4R) (Buchholz & Zempel, 2024). The loss of function, mislocalization, or toxic gain of function of pathological tau may lead to neurotoxicity and also mediate amyloid-β-induced toxicity (Wang & Mandelkow, 2016). As tau is a natively unfolded protein that doesn't tend to aggregate, mutations or post-translational modifications may lead to tau aggregation and tau-induced neurodegeneration (Gao et al., 2018). Therefore, it is important to identify brain regions in which more tau proteins are synthesized, as those regions have a higher probability of having tau pathology by simple probability.

1.3 Location of Tau aggregates

Raised phosphorylated tau levels in the cerebrospinal fluid are a biomarker for neurodegenerative diseases such as Alzheimer's disease. CSF p-tau181, p-tau217, and p-tau231 have been shown to be accurate predictors of cognitive impairment in AD patients (Ashton et al., 2022). It is commonly held that tau-pathology progression follows a distinct pattern, identified post-mortem through approximating Barack Stages. However, recent studies have concurred that this homogenous approach is limited and that individual differences lead to different progression patterns (St-Onge et al., 2023). Therefore, it is more effective to track pathological tau accumulation and spread by using individualized regions of interest (Sohn et al., 2015). In this study, we examine differences in MAPT gene expression in post-mortem brain samples from two healthy control subjects to evaluate expression differences between the temporal and visual cortices. Since the analysis only includes healthy controls, the data in this study cannot be used to reach clear conclusions on how MAPT-RNA expression affects tau pathology. The determine if a significant difference in MAPT-RNA expression, and therefore tau protein level, can be identified based on the cortex. It has been hypothesized that reducing MAPT mRNA expression and thus tau protein levels can prevent tau aggregation and could be a potential treatment option for tauopathies (Sud et al., 2014). Both Sud and colleagues and Chakravarthy and colleagues have been successful in developing antisense oligonucleotides against MAPT, which have reduced the expression of MAPT mRNA and tau levels. Lower MAPT mRNA production may reduce the production of hyperphosphorylated tau (Chakravarthy et al., 2020). Conversely, it can be theorized that brain regions with higher MAPT-RNA expression can potentially be more susceptible to tau accumulation. These can be promising sites for further research on the diagnosis and treatment of tauopathies.

1.4 Taupathology and MAPT expression

Post-translational modifications are the most prominent cause of tau aggregation (Hattiholi et al., 2025). Specifically, mutations that alter phosphorylation sites of tau may lead to hyperphosphorylation. Hyperphosphorylation of tau is three times higher in the disease state, compared to physiological conditions (Nisbet et al., 2015). Hyperphosphorylation of tau compromises microtubule stabilization and axonal transport by negatively regulating the binding of tau to microtubules. Hyperphosphorylation significantly reduces tau's affinity for microtubules (Geschwind, 2003). A previous study by Lee and colleagues has identified the entorhinal cortex

(A28/34, A35/36r) as a region with high tau-PET signaling and the first region to become tau positive, specifically in AD patients (Lee et al., 2022). Their study has also identified the inferior temporal gyri as a “propagation hub”, a brain region well-positioned to promote widespread tau aggregation (Lee et al., 2022). In light of such findings, our study examined MAPT expression in the temporal and visual cortices of healthy controls. This study aimed to compare subjects to understand if the specific identification of the inferior temporal gyri can be applied more generally to the temporal cortex. MAPT-RNA expression in the temporal cortex was compared to the visual cortex, which has not been suggested as a region with particularly high tau or as a propagation hub.

2. Procedure

2.1 Subjects

Samples were obtained from the Allen Institute’s 1,000 Gene Survey in Cortex as part of the Allen Human Brain Atlas. The Allen Institute obtained frozen tissue samples from adult male and female control or schizophrenia subjects from the brain tissue collection of the Section on Neuropathology, Clinical Disorders Branch, GCAP, IRP, National Institute of Mental Health, NIH, Bethesda, MD, and the University of Miami Brain Endowment Bank, University of Miami Miller School of Medicine, Miami, FL. All control subjects had normal neuropathological examination results, no known history of neuropsychiatric disease, and no evidence for substance use. The male and female control subjects from whose samples were tagged for MAPT-RNA were used in this study. Both of the subjects were 41 years old (Technical White Paper: In Situ Hybridization in the Allen Human Brain Atlas, 2013).

2.2 Tissue Samples

Frozen tissue samples, sectioned into coronal slabs of 1-1.5 cm thickness, were harvested by the Allen Institute. The samples from the male subject used in this study were taken from the right hemisphere, and the samples from the female subject were taken from the left hemisphere. For the male subject, 4 slices from the temporal cortex and 4 slices from the visual cortex were analyzed; for the female subject, 3 slices from the temporal cortex and 3 slices from the visual cortex were analyzed. Visual cortex samples included Brodmann’s areas 17 and 18, and temporal cortex samples included Brodmann’s areas 21 and 22 (Technical White Paper: In Situ Hybridization in the Allen Human Brain Atlas, 2013).

2.3 Image Analysis

ISH expression images obtained from the Allen Institute’s Allen Brain Atlas data portal were manually cropped on ImageJ/FIJI (NIH). Copies of the cropped images were separately thresholded for high and moderate expression levels. The cells appearing red in the original images exhibit high MAPT-RNA expression, the cells that appear green exhibit moderate MAPT-RNA expression, and the cells that appear blue exhibit low MAPT-RNA expression per ISH guidelines. Accepted red-green-blue (RGB) color model hues for high-expression (red) were 1-23, and moderate-expression (green) were 53-123. The hues were selected manually on the hue-saturation-brightness (HSB) scale to represent the colors. Low-expression (blue) cells were discarded as the blue hues were too similar to the background and could result in

misleading data. The circularity parameters used to extract the data were set at 0.70-1.00 since neuronal cell bodies are approximately circular-shaped. Percent areas, the total area of cells within the accepted hues divided by the total area of the cropped image, were calculated for each thresholded crop using FIJI.

Differences in percent areas were analyzed across different cortices and sexes for both expression levels. Statistics were run as two-way ANOVA tests on GraphPad Prism 10. The significance level was 0.05.

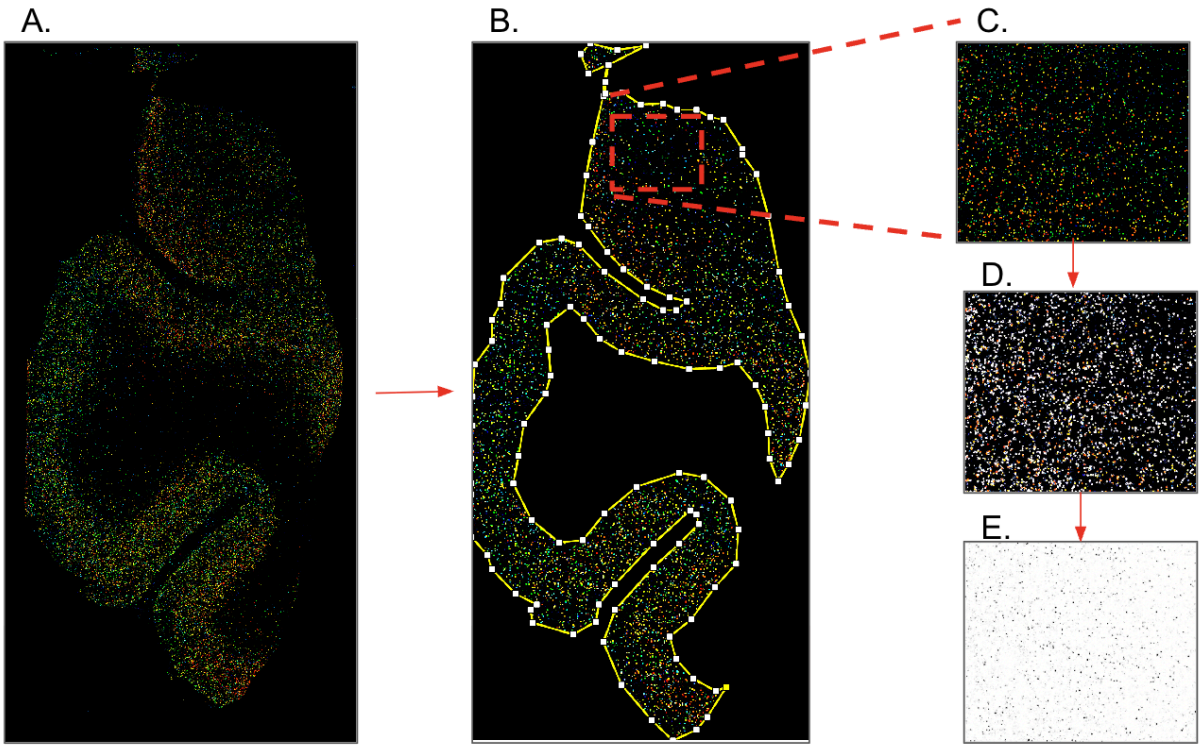

Figure 1. Image Analysis Pipeline

(A) Example ISH expression image data obtained from the Allen Institute's Allen Brain Atlas data portal.
(B) Manual cropping of the ISH expression image.
(C) Example insert to demonstrate marker expression at low, medium, and high levels.
(D) The sample was thresholded to only include the cells with the accepted green hues.
(E) Masks were obtained from the analysis of accepted cells and used for percent area calculations on FIJI.

2.4 Statistical Analysis

The extracted data were organized in Google Sheets based on subject and brain region to identify differences based on these factors. T-tests and 2-way ANOVAs were then run on GraphPad Prism 10 (San Diego, CA) for all comparisons with an alpha level of 0.05. The 2-way ANOVA test included a region effect, a subject effect, and an interaction effect. The difference between visual and temporal cortex analyses, as well as the difference between the subjects for both brain regions, was calculated. Tukey's correction was used for multiple comparisons. Importantly, the 2-ANOVA and Tukey's correction multiple comparison analyses reflect within-subject variability across samples from only one subject. Due to the small sample size of

n=1 per group, these data do not support population-level inference. The statistical tests are only descriptive. The resulting graphs from the 2-way ANOVA test were also generated on Prism 10.

Results

Figure 2 shows the percent area for high-level expression (red) cells in the samples from the visual and temporal cortices of both the male and female subjects. A 2-way ANOVA revealed a significant difference in high-level MAPT-RNA expression between the subjects ($F_{1,10} = 16.37$, p=0.002, **). However, no significant difference in expression was observed between the visual and temporal cortices within the subjects ($F_{1,10} = 0.10$, p=0.757). Post-hoc comparisons demonstrate that the male high-level MAPT expression is higher in the visual cortex compared to the temporal (p= 0.002, **).

Figure 3 shows the percent areas for medium-level expression (green) cells in the samples from the visual and temporal cortices of both the male and female subjects. A 2-way ANOVA revealed a significant difference in medium-level MAPT-RNA expression between the subjects ($F_{1,10} = 18.0$, p=0.002, **). However, no significant difference was observed between the visual and temporal cortices within the subjects ($F_{1,10} = 0.9$, p=0.345). Post-hoc comparisons demonstrate that the male medium-level MAPT expression is higher in the visual cortex compared to the temporal cortex (p<0.001, ***), and that the medium-level MAPT expression is higher for the female subject compared to the male subject in the temporal cortex. (p=0.018, *).

Figure 2. A 2-way ANOVA revealed a main effect of brain region. Post-hoc comparisons revealed a significant difference between the visual and temporal cortices in the male subject (p=0.002, **).

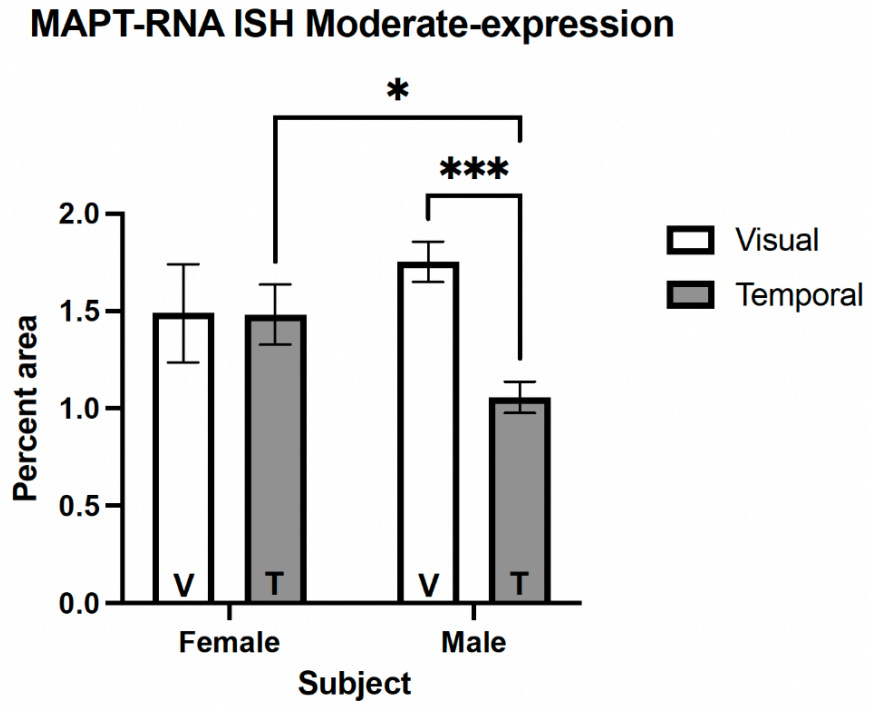

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Figure 3. A 2-way ANOVA revealed a main effect of brain region and subject. Post-hoc comparisons revealed a significant difference between the visual and temporal cortices in the male subject, and between the male and female subject in the temporal cortex (p<0.001, ***) (p=0.018, *).

Discussion

The analysis of the results shows a significant difference in high MAPT-RNA expression between the visual and temporal cortices of the male subject, and a significant difference in moderate MAPT-RNA expression between the temporal cortices of the male and female subjects and between the visual and temporal cortices of the male subject. This might be due to the low sample size or a broader lack of homogeneity in MAPT-RNA expression among individuals. Previous studies have also highlighted the heterogeneity among individuals regarding regions of tau accumulation (St-Onge et al., 2023). The differences observed within moderate MAPT expression show that the same heterogeneity can also be seen in MAPT gene expression. Although the main demographic factor separating the subjects was their sex, it is not possible to determine if the difference in expression is a sex-related or an individual difference. Similarly, the results are unlikely to be related to the difference in the hemisphere between the subjects. Especially because the sample size is not large enough for such a generalization, and there have been no previous reports of the left or right hemisphere being more susceptible to MAPT expression. Thus, more extensive studies are needed to understand the potential inter-region, inter-sex, and inter-individual differences of MAPT expression in humans, and its implications for susceptibility to tau dysfunction.

Based on Lee and colleagues' study, we hypothesized that the temporal cortex would exhibit higher expression. However, in our analysis, MAPT-RNA expression was found to be higher in the visual cortex, particularly for the male subject. A primary limiting factor in this study is the low sample size, which may be one of the reasons our findings did not align with the findings from Lee and colleagues' wider-scale study. As previously stated, the sample size is too small to make a generalization, and results may only reflect individual differences. Moreover, Lee and colleagues' study found the inferior temporal gyrus, Broadmann 20, to be a tau propagation hub. Our sample included slices from Broadmann areas 21 and 22. This may indicate that the high MAPT expression observed is specific to the inferior temporal gyrus. The results may indicate that the areas chosen for the temporal cortex samples in this study do not exhibit the same heightened MAPT expression found in the inferior temporal gyrus. In that case, it is possible that the expression in the visual cortex was higher due to individual differences, particularly given the low sample size.

Importantly, these results also do not indicate that high MAPT-RNA expression can be linked to susceptibility to tau aggregation, as only healthy controls were included in the study. This study only analyzes differences between MAPT-RNA expression, which is only directly related to tau production, not aggregation. Our aim was to identify potential sites with increased tau amount as suggestions for sites susceptible to aggregation. However, to draw a connection between tau amount and aggregation, samples from tauopathy patients would be required.

Conclusion

The findings of this study confirm that there are significant differences between different ROIs in individuals and also between individuals concerning MAPT-RNA expression. These differences suggest that it is difficult to determine potential tau accumulation sites that can be generalized to all individuals. However, identifying ROIs that are more susceptible to tau

aggregation can be a useful diagnostic tool for smaller populations or at the individual level. More extensive future studies that have higher sample sizes and that examine a wider range of brain regions are required to identify specific trends for populations.

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I would like to thank [mentor name redacted by Managing Editor] for his guidance and mentorship in developing this project, training me on the methodologies, and for reviewing and editing the paper.

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*Red is new additions/reformatting, blue has been cut/moved

Comparative Analysis of MAPT-RNA Expression Levels Across Temporal and Visual Cortices

[Author name redacted by Managing Editor]
[School name redacted by Managing Editor]
[School address redacted by Managing Editor]

Abstract

Tau aggregation and dysregulation have been associated with various neurodegenerative disorders, such as Alzheimer's Disease and various types of frontotemporal dementia. These disorders, known as taupathies, can present clinically as behavioral disorders, movement disorders, language disorders, and dementia. The heterogeneity of clinical presentation among tauOpathies and the lack of reliable biomarkers for early diagnosis hinder the ability to diagnose tauopathies before they have reached stages at which they are mostly untreatable. For most of these disorders, the primary brain region of hyperphosphorylated, or mutant tau accumulation, has not been identified. Higher expression of MAPT-RNA is more likely to lead to higher deposition and potentially accumulation of mutant tau; therefore, identifying regions with high levels of MAPT-RNA could help identify potential sites of tau accumulation. Although significant heterogeneity in regions of interest (ROI) of MAPT-RNA expression and tau accumulation has been found, identifying potential ROIs that can be generalized to small populations can be valuable in diagnosis and treatment. This study is a small-scale exploratory analysis of the expression locations of the human MAPT gene RNA in post-mortem brain samples from two healthy controls, aimed at seeing if the temporal or visual cortex is comparatively more susceptible toor even individuals can serve as a diagnostic tool in taupathies. By identifying a primary region of accumulation, check-ups and early diagnosis may be made available to those affected by risk factors for taupathies. Therefore, this study will analyze the expression locations of the human MAPT gene RNA in post-mortem brain samples from two healthy controls to determine if a cortical region with specifically high MAPT-RNA expression can be identified and suggested as a potential ROI for tau accumulation.
Keywords: MAPT, tau, frontotemporal dementia, MAPT-RNA expression, tauopathy, tau accumulation

1. Introduction

1.1 The Human MAPT Gene

The human MAPT gene, which encodes for tau protein, is located on chromosome 17q21.31. The mRNA transcripts of the MAPT gene undergo alternative splicing in exons 2, 3, and 10, leading to 6 tau isoforms in the central nervous system (Buchholz & Zempel, 2024). There have been links found between more than 50 MAPT mutations and various neurodegenerative disorders such as frontotemporal dementia with Parkinsonism-17 (FTDP17), corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), Pick's disease, and Alzheimer's disease (AD) (Strang et al., 2019). Understanding the expression of the MAPT gene

is important for understanding the regulation of tau protein levels, which can lead to neurodegenerative disorders if found in excess.

1.2 Tau Proteins

Tau proteins are a part of the microtubule-associated protein (MAP) family. In humans, tau proteins are most commonly present in neuronal cells, though smaller amounts are also found in peripheral tissues such as the heart, kidney, and lung (Xu et al., 2025). In the neuron, tau is abundant in the axons, though it is also present in smaller quantities in dendrites. Under physiological conditions, the tau protein's primary functions are stabilizing neuronal microtubules, facilitating axonal transportation, and modulating synaptic plasticity (Buchholz & Zempel, 2024). There are 6 brain-specific isoforms of the tau protein. These isoforms are differentiated by the number of amino-terminal inserts (0N, 1N, or 2N) or carboxyl-terminal repeat domains (3R or 4R) (Buchholz & Zempel, 2024). The loss of function, mislocalization, or toxic gain of function of pathological tau may lead to neurotoxicity and also mediate amyloid-β-induced toxicity (Wang & Mandelkow, 2016). As tau is a natively unfolded protein that doesn't tend to aggregate, mutations or post-translational modifications may lead to tau aggregation and tau-induced neurodegeneration (Gao et al., 2018). Therefore, it is important to identify brain regions in which more tau proteins are synthesized, as those regions have a higher probability of having tau pathology by simple probability. The present study evaluates MAPT-RNA expression to identify predicted regions of high tau deposition.

1.3 Location of Tau aggregates

Raised phosphorylated tau levels in the cerebrospinal fluid are a biomarker for neurodegenerative diseases such as Alzheimer's disease. CSF p-tau181, p-tau217, and p-tau231 have been shown to be accurate predictors of cognitive impairment in AD patients (Ashton et al., 2022). However, obtaining CSF tau measurements requires an invasive spinal tap procedure and does not provide significant information on the spatial distribution of tau (Dani et al., 2016). Moreover, potential treatments for diagnoses provided by spinal taps are limited when spatial location of tau dysfunction is not known. Though it is commonly held that tau-pathology progression follows a distinct pattern, identified post-mortem through approximating Barack Stages. However, recent studies have concurred that this homogenous approach is limited and that individual differences lead to different progression patterns (St-Onge et al., 2023). Therefore, it is more effective to track pathological tau accumulation and spread by using individualized regions of interest (Sohn et al., 2015). In this study, we examine differences in MAPT gene expression in post-mortem brain samples from two healthy control subjects to evaluate expression differences between the temporal and visual cortices. Since the analysis only includes healthy controls, the data in this study cannot be used to reach clear conclusions on how MAPT-RNA expression affects tau pathology. The determine if a significant difference in MAPT-RNA expression can be identified based on the cortex. It has been hypothesized that reducing MAPT mRNA expression and thus tau protein levels can prevent tau aggregation and could be a potential treatment option for tauopathies (Sud et al., 2014). Both Sud and colleagues and Chakravarthy and colleagues have been successful in developing antisense oligonucleotides against MAPT, which have reduced the expression of MAPT mRNA

and tau levels. Lower MAPT mRNA production may reduce the production of hyperphosphorylated tau (Chakravarthy et al., 2020). Conversely, it can be theorized that brain regions with higher MAPT-RNA expression can potentially be more susceptible to tau accumulation. These can be promising sites for further research on the diagnosis and treatment of tauopathies.

1.4 Taupathology and MAPT expression

Post-translational modifications are the most prominent cause of tau aggregation (Hattiholi et al., 2025). Specifically, mutations that alter phosphorylation sites of tau may lead to hyperphosphorylation. Hyperphosphorylation of tau is three times higher in the disease state, compared to physiological conditions (Nisbet et al., 2015). Hyperphosphorylation of tau compromises microtubule stabilization and axonal transport by negatively regulating the binding of tau to microtubules. Hyperphosphorylation significantly reduces tau's affinity for microtubules (Geschwind, 2003). A previous study by Lee and colleagues has identified the entorhinal cortex (A28/34, A35/36r) as a region with high tau-PET signaling and the first region to become tau positive, specifically in AD patients (Lee et al., 2022). Their study has also identified the inferior temporal gyri as a "propagation hub", a brain region well-positioned to promote widespread tau aggregation (Lee et al., 2022). In light of such findings, our study examined MAPT expression in the temporal and visual cortices of healthy controls. This study aimed to compare subjects to understand if the specific identification of the inferior temporal gyri can be applied more generally to the temporal cortex. MAPT-RNA expression in the temporal cortex was compared to the visual cortex, which has not been suggested as a region with particularly high tau or as a propagation hub.

2. Procedure

2.1 Subjects

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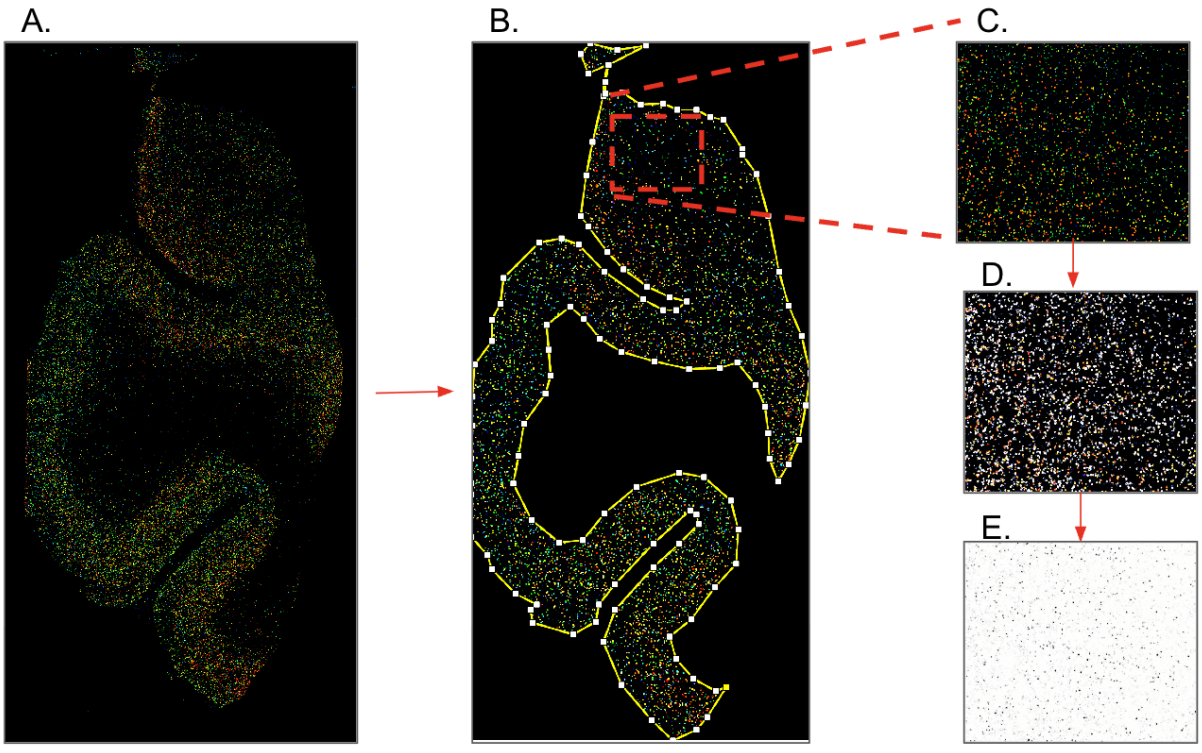

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Results

Figure 2 shows the percent area for high-level expression (red) cells in the samples from the visual and temporal cortices of both the male and female subjects. A 2-way ANOVA revealed a significant difference in high-level MAPT-RNA expression between the visual and temporal cortex in the male subject ($F_{1,10} = 16.37$, p=0.002). However, no significant difference was observed between the visual and temporal cortices in the female subject (p=0.891). There was no significant difference based on subject or sex-related differences in the expression of MAPT-RNA in the visual ($F_{1,10} = 0.8$, p=0.156) or the temporal cortex ($F_{1,10} = 0.8$, p=0.296). Post-hoc comparisons demonstrate that the male high-level MAPT expression is higher in the visual cortex compared to the temporal (p= 0.002, **).

Figure 3 shows the percent areas for medium-level expression (green) cells in the samples from the visual and temporal cortices of both the male and female subjects. A 2-way ANOVA revealed a significant difference in medium-level MAPT-RNA expression between the visual and temporal cortex in the male subject (p<0.001$F_{1,10} = 18.0$, p=0.002, **). However, no significant difference was observed between the visual and temporal cortices in the female subject (p>0.999) ($F_{1,10} = 0.9$, p=0.345). The 2-way ANOVA also revealed a significant difference in medium-level MAPT-RNA expression in the temporal cortex between the male and female subjects (p=0.018) ($F_{1,10} = 18.0$, p=0.002). No significant difference was observed in the visual cortex between the male and female subjects (p=0.165). Post-hoc comparisons demonstrate that the male medium-level MAPT expression is higher in the visual cortex compared to the temporal cortex (p<0.001, ***), and that the medium-level MAPT expression is higher for the female subject compared to the male subject in the temporal cortex. (**, p<0.01).

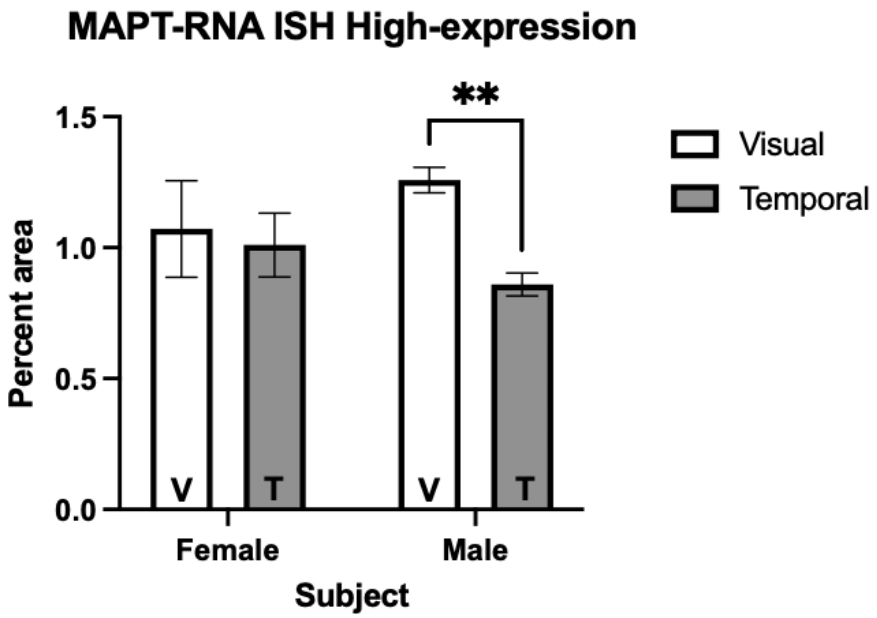

Figure 2. A 2-way ANOVA revealed a main effect of brain region. Post-hoc comparisons revealed a significant difference between the visual and temporal cortices in the male subject (p=0.002, **).

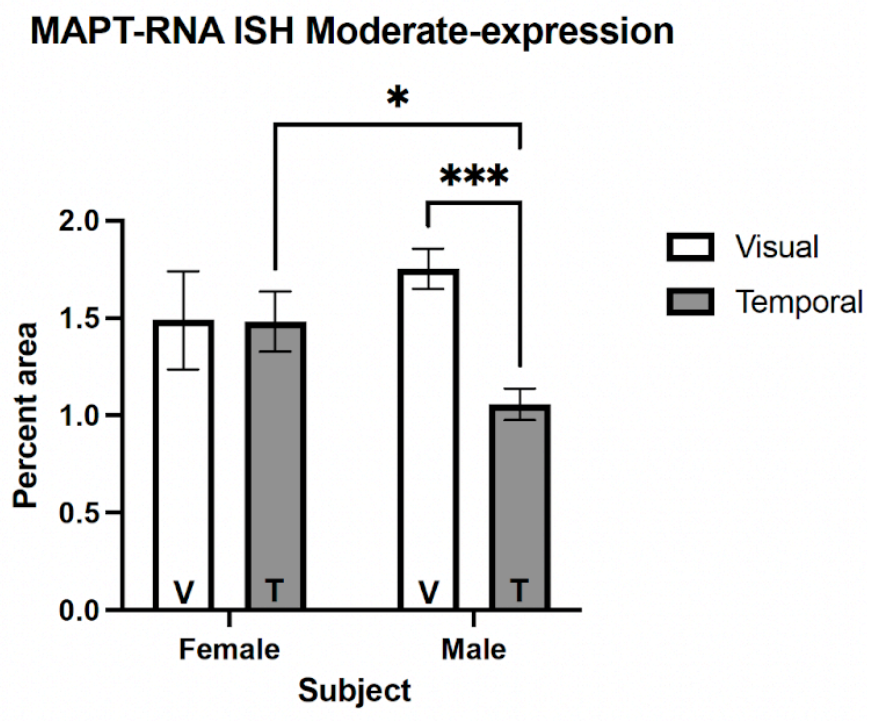

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Figure 3. A 2-way ANOVA revealed a main effect of brain region and subject. Post-hoc comparisons revealed a significant difference between the visual and temporal cortices in the male subject, and between the male and female subject in the temporal cortex (p<0.001, ***) (p=0.018, *).

Discussion

The analysis of the results shows a significant difference in high MAPT-RNA expression between the visual and temporal cortices of the male subject, and a significant difference in moderate MAPT-RNA expression between the temporal cortices of the male and female subjects and between the visual and temporal cortices of the male subject. There is no trend in results that could be observed in both subjects in either high or moderate expression. This might be due to the low sample size or a broader lack of homogeneity in MAPT-RNA expression among people. Previous studies have also highlighted the heterogeneity among individuals regarding regions of tau accumulation (St-Onge et al., 2023). The differences observed within moderate MAPT expression show that the same heterogeneity can also be seen in MAPT gene expression. Though the main demographical factor separating the subjects was their sex, this finding is more likely caused by individual differences rather than a difference between sexes in general. Similarly, the results are unlikely to be related to the difference in the hemisphere between the subjects, as the sample size is not high enough for a generalization, and there have been no previous reports of the left or right hemisphere being more susceptible to tau aggregation or MAPT expression. Thus, more extensive studies are needed to understand the potential inter-region, inter-sex, and inter-individual differences of MAPT expression in humans, its implications for susceptibility to tau dysfunction.

Based on Lee and colleagues' study, we hypothesized that the temporal cortex would exhibit higher expression. However, in our analysis, MAPT-RNA expression was found to be higher in the visual cortex, particularly for the male subject. A primary limiting factor in this study is the low sample size, which may be one of the reasons our findings did not align with the findings from Lee and colleagues' wider-scale study. As previously stated, the sample size is too small to make a generalization, and results may only reflect individual differences. Moreover, Lee and colleagues' study found the inferior temporal gyrus, Broadmann 20, to be a tau propagation hub. Our sample included slices from Broadmann areas 21 and 22. This may indicate that the high MAPT expression observed is specific to the inferior temporal gyrus. The results may indicate that the areas chosen for the temporal cortex samples in this study do not exhibit the same heightened MAPT expression found in the inferior temporal gyrus. In that case, it is possible that the expression in the visual cortex was higher due to individual differences, particularly given the low sample size.

Importantly, these results also do not indicate that high MAPT-RNA expression can be linked to susceptibility to tau aggregation, as only healthy controls were included in the study. This study only analyzes differences between MAPT-RNA expression, which is only directly related to tau production, not aggregation. Our aim was to identify potential sites with increased tau amount as suggestions for sites susceptible to aggregation. However, to draw a connection between tau amount and aggregation, samples from tauopathy patients would be required.

Conclusion

The findings of this study confirm that there are significant differences between different ROIs in individuals and also between individuals concerning MAPT-RNA expression. These

differences suggest that it is difficult to determine potential tau accumulation sites that can be generalized to all individuals. However, identifying ROIs that are more susceptible to tau aggregation can be a useful diagnostic tool for smaller populations or at the individual level. More extensive future studies that have higher sample sizes and that examine a wider range of brain regions are required to identify specific trends for populations.

Acknowledgments

I would like to thank [mentor name redacted by Managing Editor] for his guidance and mentorship in developing this project, training me on the methodologies, and for reviewing and editing the paper.

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Major Comments:

- Introduction
  - The introduction should have more citations throughout 👍🏽

I had grouped many of the citations for more backbone information. I added more citations and three more papers for variety.

  - "The object of this study is to determine if a significant difference in MAPT-RNA expression can be identified based on the cortex." - This part of the paper frames the objective of your study and the research question. Thus, it should more clearly lay out: "This study is a small-scale exploratory analysis of the expression locations of the human MAPT gene RNA in post-mortem brain samples from two healthy controls, aimed at seeing if the temporal or visual cortex is comparatively more susceptible to tau accumulation." I hope this expresses the objective more clearly. I included it in the Abstract but I could integrate it in the Introduction if you think that would be better.

  - Why have you chosen to compare temporal and visual cortices? 👍🏽

"This study aimed to compare subjects to understand if the specific identification of the inferior temporal gyri can be applied more generally to the temporal cortex. MAPT-RNA expression in the temporal cortex was compared to the visual cortex, which has not been suggested as a region with particularly high tau or as a propagation hub." Introduction 1.4

  - What would a significant difference between these cortices in MAPT-RNA expression point to? 👍🏽

"... it can be theorized that brain regions with higher MAPT-RNA expression can potentially be more susceptible to tau accumulation. These can be promising sites for further research on the diagnosis and treatment of tauopathies." Introduction 1.3

  - Given your analysis is of health controls, how does this information inform us on tauopathies? 👍🏽

I included tauopathies in my paper to show the significance of identifying sites of high MAPT-RNA expression and tau deposition. I tried to include a clearer explanation of how using healthy controls would not inform us about tauopathy pathology at the end of the Discussion. However, I am worried that it comes too late in the paper, please let me know if you think it would be better to move it to the Introduction.

  - The research question is phrased as looking for whether there is a "significant difference" – which could sound mechanical and is better phrased scientifically

I am a little confused about this comment. I meant statistically significant difference, but that might not be appropriate terminology.

- Lay out the rationale more clearly for why a difference in the MAPT-RNA expression of these cortices at baseline (in healthy individuals) should theoretically indicate which regions are more vulnerable to tau pathology and thus potential sites of early identification in tauopathies

"Lower MAPT mRNA production may reduce the production of hyperphosphorylated tau (Chakravarthy et al., 2020). Conversely, it can be theorized that brain regions with higher MAPT-RNA expression can potentially be more susceptible to tau accumulation. These can be promising sites for further research on the diagnosis and treatment of tauopathies."

I tried to connect them here, but I am not sure it is a strong enough justification. As I said above, I understand that we can't make a strong connection, especially by just using healthy controls, so maybe I could include that explanation here?

-

Methods:

- Why were those hue/circularity thresholds chosen? Cite previous literature or explain how this was chosen. 👍🏽

"The hues were selected manually on the hue-saturation-brightness (HSB) scale to represent the colors."

"The circularity parameters used to extract the data were set at 0.70-1.00 since neuronal cell bodies are approximately circular-shaped."

The hue and circularity ranges were Dr. Avila's suggestions. I couldn't find any other literature to cite, so I tried to explain it like so. Please let me know if there is a better way to express that.

- Explicitly state what the independent and dependent variables are in the 2 way ANOVA - not just "column" and "row" 👍🏽

"The 2-way ANOVA test included a region effect, a subject effect, and an interaction effect."

2.4 Statistical Analysis

- The text does acknowledge this but statistical testing (2-way ANOVA and post-hoc Tukey) is not meaningful with n=1 per group. These tests assume multiple independent samples per condition, not multiple slices from the same individual. Thus, the paper should clarify that the analyses reflect within-subject variability across slices, not population-level inference and that statistical tests here are descriptive. 👍🏽

"Importantly, the 2-ANOVA and Tukey's correction multiple comparison analyses reflect within-subject variability across samples from only one subject. Due to the small sample size of n=1 per group, these data do not support population-level inference. The statistical tests are only descriptive."
2.4 Statistical Analysis

- There is some redundancy in the last two sections of the Methods. This could be condensed into one section or perhaps make one into a Statistical Analysis section. 👍🏽
2.4 Statistical Analysis

- Results:
  - The reporting of 2-Way ANOVA is confusing: "A 2-way ANOVA revealed a significant difference in high-level MAPT-RNA expression between the visual and temporal cortex in the male subject (16.37, p=0.002). However, no significant difference was observed between the visual and temporal cortices in the female subject (p=0.891)." – a 2-way ANOVA cannot tell the difference is within the male as it analyzes across both. Did you mean that there was a significant difference between cortices (across both subjects) but that this was stronger in males in post-hoc tests? Or the interaction of sex and cortex was significant? 👍🏽
  - Make sure reporting of statistics is consistent: ensure each result is accompanied by F/T-statistics and p-value, and that the test you used is explicitly stated. Also, some stats have ** and some do not. 👍🏽

I rewrote most of the results section to be more consistent and to interpret the ANOVA and Multiple Comparisons results correctly. However, I am still not entirely sure about them. Could you please let me know if I am misinterpreting something if I attach photos of the Prism ANOVA results?

- Discussion
  - "Though the main demographical factor separating the subjects was their sex, this finding is more likely caused by individual differences rather than a difference between sexes in general." – The current analysis cannot differentiate these two sources of variation. I recommend to either cite previous work showing no sex differences or phrase it is not possible to determine if this is sex or individual differences. 👍🏽

"Although the main demographic factor separating the subjects was their sex, it is not possible to determine if the difference in expression is a sex-related or an individual difference."

Discussion 1st paragraph

- "Their study has also identified the inferior temporal gyri as a "propagation hub", a brain region well-positioned to promote widespread tau aggregation (Lee et al., 2022). In light of such findings, our study examined MAPT expression in the temporal and visual cortices of healthy controls." – This should be mentioned earlier in the paper. Did this lead you to have a hypothesis that the temporal regions would have more MAPT-RNA compared to visual? If so, this should be made clear in the intro and you should discuss how you found the opposite and consider why. 👍🏽

I moved the first part to Introduction 1.4

"This study aimed to compare subjects to understand if the specific identification of the inferior temporal gyri can be applied more generally to the temporal cortex. MAPT-RNA expression in the temporal cortex was compared to the visual cortex, which has not been suggested as a region with particularly high tau or as a propagation hub."

"Moreover, Lee and colleagues' study found the inferior temporal gyrus, Broadmann 20, to be a tau propagation hub. Our sample included slices from Broadmann areas 21 and 22. This may indicate that the high MAPT expression observed is specific to the inferior temporal gyrus. The results may indicate that the areas chosen for the temporal cortex samples in this study do not exhibit the same heightened MAPT expression found in the inferior temporal gyrus. In that case, it is possible that the expression in the visual cortex was higher due to individual differences, particularly given the low sample size."

Discussion paragraph 2

- The phrasing implies too much causality at times. For example: "A specific region that had significantly increased MAPT expression would be more likely to have higher tau expression and to be a potential "propagation hub". MAPT-RNA expression does not equal tau aggregation and so this should be more cautiously phrased.

I removed that specific sentence. I think the end of Introduction 1.4 explains that idea with more evidence and cautious language, but please let me know if you disagree.

- "Post-translational modifications are the most prominent cause of tau aggregation (Hattiholi et al., 2025). Specifically, mutations that alter phosphorylation sites of tau may lead to hyperphosphorylation. Hyperphosphorylation of tau is three times higher in the disease state, compared to physiological conditions (Nisbet et al., 2015).

Hyperphosphorylation of tau compromises microtubule stabilization and axonal transport by negatively regulating the binding of tau to microtubules.

Hyperphosphorylation significantly reduces tau's affinity for microtubules (Geschwind, 2003)." – This section would be more appropriate in the introduction 👍🏽
I have moved both of these to Introduction 1.4

Minor comments:

- "Taupathies" should be "tauopathies" throughout the paper 👍🏽All of them have been corrected
- Abstract: "Higher expression of MAPT-RNA is more likely to lead to higher deposition and potentially accumulation of mutant tau; therefore, identifying regions with high-tau expression can be a valuable approach to identifying potential sites of tau accumulation." This sentence is unclear - do you mean to say mapping regions with high levels of MAPT-RNA could help identify potential sites of tau accumulation. 👍🏽
I rewrote the sentence as "Higher expression of MAPT-RNA is more likely to lead to higher deposition and potentially accumulation of mutant tau; therefore, mapping regions with high levels of MAPT-RNA could help identify potential sites of tau accumulation." I hope that works better

- Abstract: "Though significant heterogeneity in regions of interest (ROI) of MAPT-RNA expression and tau accumulation has been found, investigating potential ROIs that can be generalized to small populations or even individuals can serve as a diagnostic tool in taupathies." Meaning of this sentence is unclear.
"Although significant heterogeneity in regions of interest (ROI) of MAPT-RNA expression and tau accumulation has been found, identifying potential ROIs that can be generalized to small populations can be valuable in diagnosis and treatment." I slightly rephrased this sentence to make it clearer but I am not sure if it fixed the issue, let me know if you would like for me to rewrite it.

- Abstract" Therefore, this study will analyze the expression locations of the human MAPT gene RNA in post-mortem brain samples from two healthy controls to determine if a cortical region with specifically high MAPT-RNA expression can be identified and suggested as a potential ROI for tau accumulation." This should be in past tense and might be good to frame it as a small exploratory analysis to avoid overstating the generalizability 👍🏽
I rewrote it as "This study is a small-scale exploratory analysis of the expression locations of the human MAPT gene RNA in post-mortem brain samples from two healthy controls, aimed at seeing if the temporal or visual cortex is comparatively more susceptible to tau accumulation."

- Introduction: Section 1.3 is missing 👍🏽 This was just a formatting mistake; I added the section title

- Introduction: “There have been links found between more than 50 MAPT mutations and various neurodegenerative disorders such as frontotemporal dementia with Parkinsonism-17 (FTDP17), corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), Pick’s disease, and Alzheimer’s disease (AD).” This sentence has no citation. It may also be helpful to provide information about what changes to tau biology are associated with some of these mutations
I added the citation (Strang et al., 2019). Changes in tau biology that lead to disease are included at the start of Introduction 1.4, please let me know if you would like me to move them here.

Author 100108, Submission 100101: ***"Comparative Analysis of MAPT-RNA Expression Levels Across Temporal and Visual Cortices."***

The revisions are thoughtful, thorough, and clearly strengthen the manuscript. The introduction is now much better supported with citations. Your revised framing of the study objective in the Abstract is also much improved and more accurate. I agree adding a similar sentence in the Introduction (maybe right after describing the gap in identifying early tau-vulnerable regions) would better orient the reader.

You appropriately add context to why you chose to compare temporal and visual cortices and what results would therefore imply. Additionally, I think you have now added enough information on the limitations for what conclusions can be drawn from a healthy control. I don't think it comes too late – as you mentioned in your response - since you have included the following in the introduction: "Since the analysis only includes healthy controls, the data in this study cannot be used to reach clear conclusions on how MAPT-RNA expression affects tau pathology." It is standard practice to expand further on limitations in the discussion, so how you have it is strong.

There is now a typo in the following sentence: "**The** determine if a significant difference in MAPT-RNA expression, and therefore tau protein level, can be identified based on the cortex." My previous comment about mentioning statistical significance in your research question/objective was to note that as scientists our goal should be to determine if a difference actually exists, and statistical tests help us measure that. So instead of saying you aim to "determine if a significant difference exists" you might say you aim to establish if "regional differences in MAPT-RNA expression exist at baseline …etc". I hope this is clearer, but it is also acceptable to leave it as you have it.

Your Methods section is also much improved. Given that the hue and circularity thresholds were selected in consultation with your mentor rather than based on published convention, your current phrasing is appropriate. Additionally, the Statistics are now much better described in their own section.

Your new phrasing correctly emphasizes that the ANOVAs reflect *within-subject variability*, not group-level inference. In your response to the reviews, you mentioned you are unsure about your 2-way ANOVA results. The way it is written now is much better and sounds accurate to me: "A 2-way ANOVA revealed a significant difference in high-level MAPT-RNA expression between the subjects ($F_{1,10} = 16.37$, p=0.002, **). However, no significant difference in expression was observed between the visual and temporal cortices within the subjects ($F_{1,10} = 0.10$, p=0.757). Post-hoc comparisons demonstrate that the male high-level MAPT expression is higher in the visual cortex compared to the temporal (p= 0.002, **)."

Overall, excellent work and thank you for a thorough response!

Recommendation: Accept with minor revisions