Frontotemporal Degeneration Pathological Astrocyte Morphology Identification for Deep Learning Algorithms

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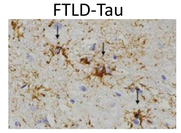
Introduction

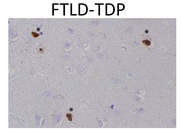
FTD is a clinical variant of frontotemporal lobar degeneration (FTLD). The FTLD spectrum is a heterogeneous group of neurodegenerative diseases that affects the frontal and temporal lobes of the brain. FTLD pathology is primarily comprised of FTLD-Tau or FTLD-TDP.[1] Due to the considerable heterogeneity of neurodegenerative diseases, current diagnostic techniques and clinical criteria cannot reliably predict the underlying neuropathology. Biomarker discovery is essential to reveal the etiology for therapeutic development and future clinical trials. Reactive astrogliosis, defined as astrocytic activation due to CNS damage, is a hallmark of FTLD and indicates neurodegeneration.[2] While glial fibrillary acidic protein (GFAP) is ubiquitous among astrocytes, it is only detectable in reactive astrocytes due to increase GFAP translation.[3] Therefore, staining for GFAP in neurodegenerative brain tissue allows us to identify astrocytes. However, there is a lack of research investigating different astrocyte morphologies involved in FTLD, such as interlaminar, protoplasmic, fibrous, and vessel processes. Our study sought to address this by examining astrocyte morphology.

Objective: Stain FTD brain tissue for GFAP to identify different astrocyte morphologies to create training data for deep learning algorithm.

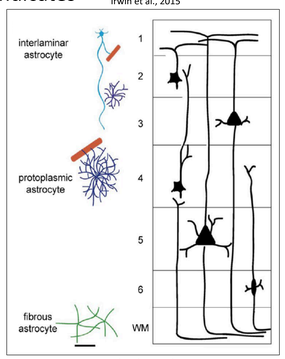
FTLD-Tau



FTLD-TDP



Irwin et al., 2015



Astrocyte morphology locations in relation to neocortical levels.[4]

Qualitative Results

Astrocyte Morphologies in GFAP-stained tissue (marked by red arrow)

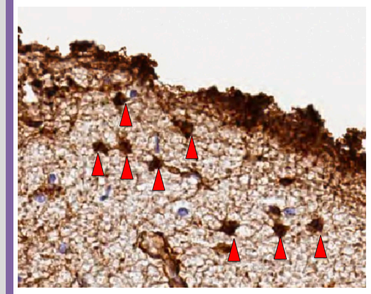


Figure 1. Interlaminar astrocytes Morphologic criteria: located in layer I, spheroid somas, short processes.[4,5]

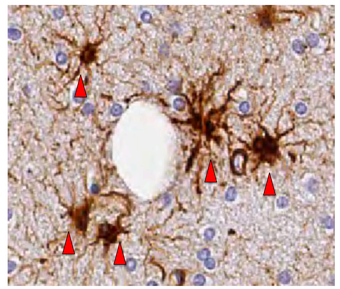


Figure 2. Protoplasmic astrocytes Criteria: located in layers II-VI, star-shaped, end feet contact vessels, large soma .[4,6]

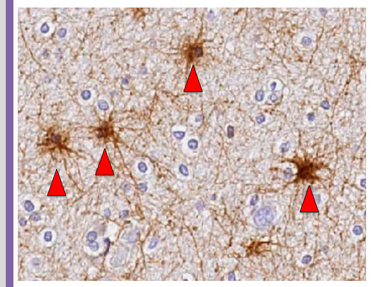


Figure 3. Fibrous astrocytes Criteria: located in white matter, longer processes than protoplasmic, spatial domains overlap .[4,6]

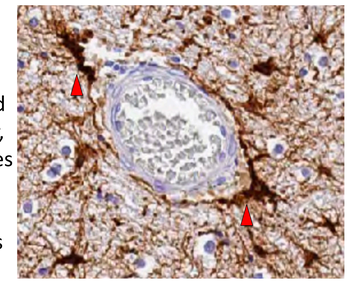


Figure 4. Astrocytic processes surrounding vessels Astrocytes are known for Blood-Brain Barrier regulation by enveloping blood vessels .[4]

Conclusion

Summary

Here, we propose a comprehensive digital histology image analysis pipeline for different GFAP stained astrocytic morphologies in FTLD human brain tissue. Our pipeline uses machine learning, to increase the reliability and statistical power of future studies.

Limitations

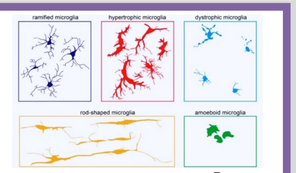
- There is a lack of consensus on classification of astrocyte morphologies.
- GFAP upregulation can lead to the false impression of an increase in astrocyte numbers.[6]

Applications

- High-throughput methods to elucidate FTLD neuropathology.
- Astrocytes as indicators of neurodegeneration and CSF biomarkers for FTLD diagnosis.
- Deep learning improves efficiency of data acquisition, providing a larger dataset thereby advancing clinical trial development.

Future Directions

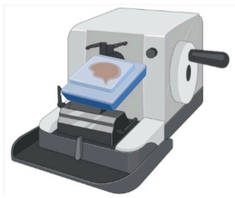
- Complete and validate training annotations.
- Compare microglia morphological differences to astrocyte morphologies.



Microglia morphologies.[7]

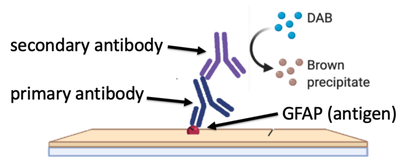
Methods

Tissue Preparation



We obtained blocks of FTD brain tissue from the Center for Neurodegenerative Disease Research (CNDR). Then, we utilized a microtome to cut the brain tissue and subsequently mounted onto slides.

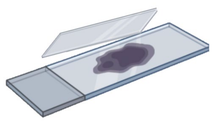
Immunohistochemistry (IHC)



Stained the tissue for GFAP to identify astrocytes following the IHC procedure:

1. Deparaffinization/Rehydration
2. Peroxidase Block
3. Application of primary antibody
4. Incubate for 16-20 hours
5. Application of secondary antibody
6. Application of Avidin-biotin complex
7. Counterstain: Hematoxylin
8. Dehydration

Digital Whole Slide Images



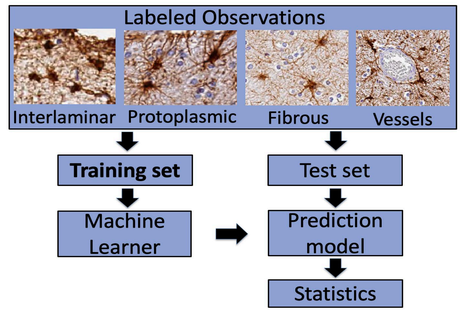
ITK-SNAP PICSL Histology Annotator

The slides were cover slipped and scanned to create digital whole slide images. The images were uploaded onto ITK-SNAP PICSL Histology Annotator (designed by Dr. Paul Yushkevich) which had a specific class for each astrocyte morphology of interest: interlaminar, protoplasmic, fibrous, processes surrounding vessels.

Deep Learning

Images were annotated by class, to provide training data for the supervised deep learning algorithm, WILDCAT. The training data will be validated and applied to a larger dataset to automatically detect and quantify astrocytes.

Supervised Learning Diagram:



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