Exploring the Relationship between Spatial Transcriptomics and Metal Accumulation in Amyotrophic Lateral Sclerosis

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ABSTRACT

- ALS (Amyotrophic Lateral Sclerosis) is a neurodegenerative disease that affects nerve cells in the brain as well as the spinal cord. Over time it leads to loss of muscle control, as the motor neurons that control voluntary muscles die.
- Accumulations of metals such as iron , zinc, and copper have been associated with ALS, and thus elemental imaging is a potential avenue for diagnoses and understanding the genetic and biochemical pathways underlying the disease.
- Our research explores the relationship between tissue morphology, spatial transcriptomic data, and metal accumulation in cases of amyotrophic lateral sclerosis(ALS)

INTRODUCTION

- ALS targets pyramidal Betz cells in the cerebral cortex and the loss of bulbar and spinal cord motoneurons, leading to cell death via apoptosis or regulated cell death.
- Roughly 5,000 patients are diagnosed annually in the United States, with a typical prognosis of two to five years of life expectancy accompanying diagnosis
- Manganese, zinc, copper concentrations have been observed to be elevated in ALS patients, demonstrating a potential neurotoxic relationship, and furthermore may affect gene expression

Coregistration METHODS

Coregistration Pipeline (ST)

1. Annotated predicted subsection of intact WSI using qupath, making sure to match relative dimensions
2. Cropped and masked the annotated subsection using a qupath script
3. Coregistered annotated subsection with subsection of intact WSI (ROI)
4. Warped coordinates onto the annotated subsection

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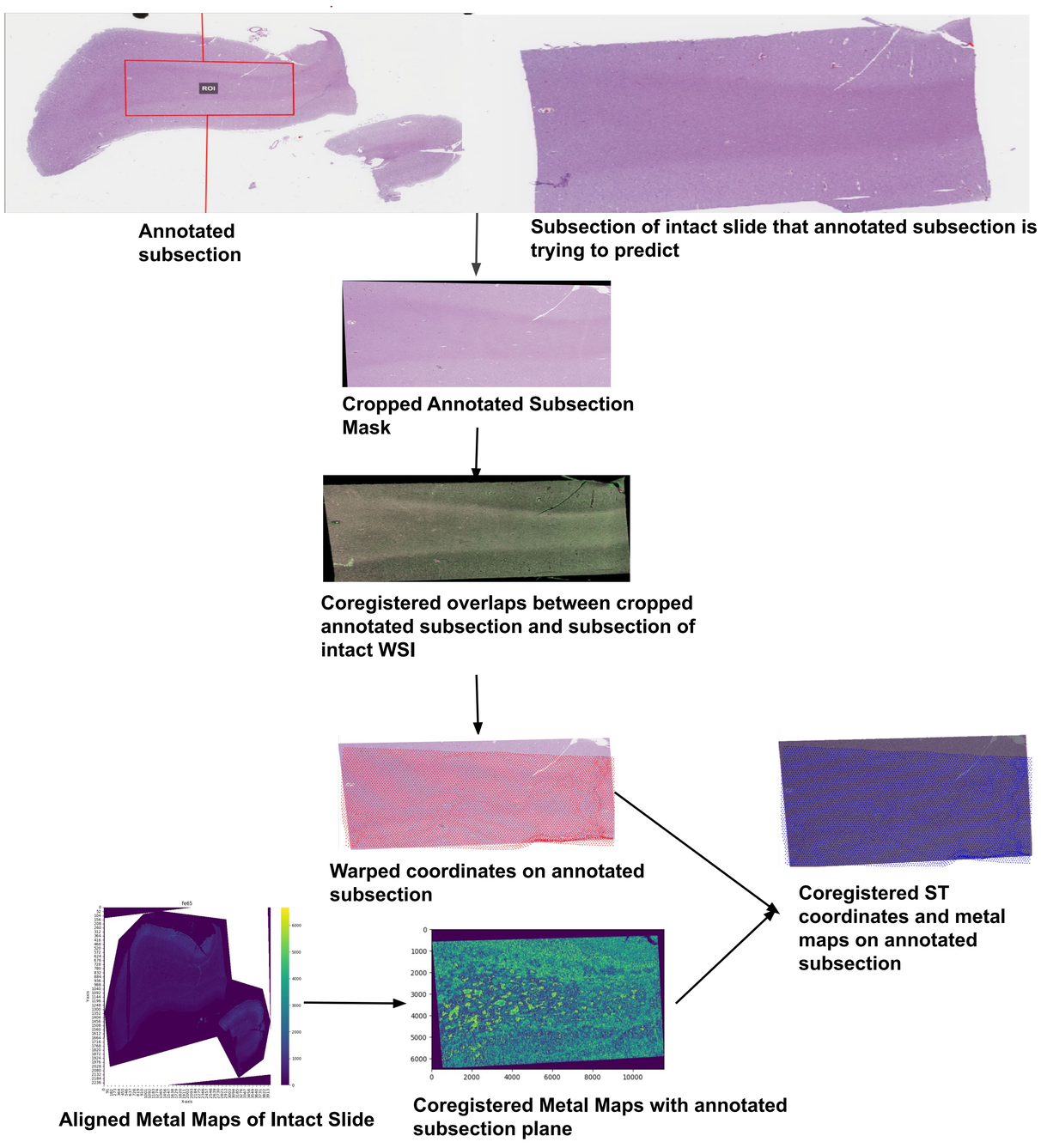
Coregistration Pipeline (Metal Maps)

1. Aligned metal maps to intact WSI using the application TRACE
2. Transformed aligned metal maps to the coordinate plane of the annotated subsection

Figure 1



Pathway Analysis METHODS

1. The average metal concentration for each Visium spot was found by assigning each pixel in a metal map to the nearest Visium spot via the nearest neighbors algorithms, and then averaging all of the metal abundance values corresponding to each Visium spot.
2. The top 47 genes based on gene expression values were used to generate a Spearman correlation matrix (between gene expressions and average metal abundances), which provided p-values for copper, zinc, manganese, magnesium, and iron
3. For each metal, enriched gene pathway analysis was run to determine which genetic pathways were associated with the genes correlated to metal concentration.

Results of Pathway Analysis

- In figures 3 and 4, manganese and magnesium have statistically significant correlation with an adjusted p-value <0.05 for pathways associated with inhibitory and excitatory neurotransmitters such as acetylcholine, serotonin, norepinephrine, glutamate, dopamine, and gamma-aminobutyric acid(GABA).
- Figure 5 depicts genetic pathways for copper, and the sole genetic pathway observed was that of long-term potentiation. However, the p-value hovered slightly under 0.05, increasing uncertainty regarding the statistical significance of this relationship
- In figures 6 and 7, zinc and iron was seen to be associated with pathways for the response to elevated platelet systolic Ca2+, which is associated with phospholipase-C[7].

Figure 3

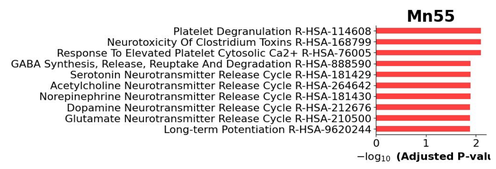


Figure 4

Figure 5

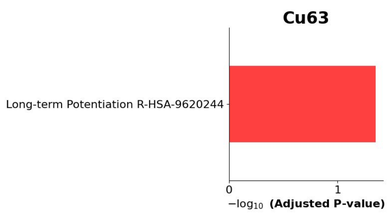


Figure 6

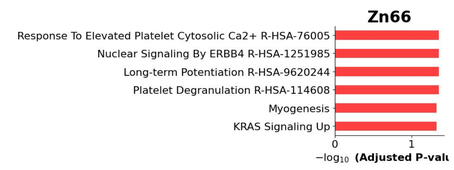
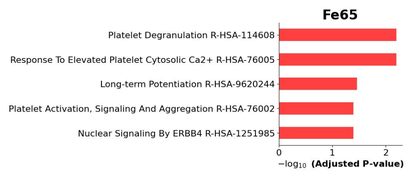


Figure 7



Discussion

- Enriched gene pathway analysis found multiple correlations with statistical significance and a plausible relationship to the pathogenesis of ALS
- The strong association between the concentration of manganese and magnesium and genetic pathways for a variety of neurotransmitters suggest that may inhibit signaling pathways and thus may accelerate the death of motor neurons in ALS patients
- The pathway for long-term potentiation(LTP)—the strengthening of synapses—was seen to be associated with genes correlated with copper. LTP is important for strengthening of synapses and is considered crucial for learning. Reduced LTP may be correlated to ALS. Research has shown that copper can have both inhibitory and necessary roles in LTP showing the metal's potential significance on ALS diagnosis.
- the association between zinc and iron and pathways associated with phospholipase-C suggest that they play a role in signal transduction pathways for neurotransmitters in brainstem motor neurons, potentially inhibiting the transmission of neurotransmitters from motoneurons and thus limiting their function.

Conclusion

- The research conducted shows the potential use of copper, zinc, manganese, magnesium, and iron for ALS diagnosis
- Future work includes building an ensemble model,that may be constructed to predict metal ion concentrations and aid in diagnosing and understanding ALS. Furthermore, a more accurate coregistration pipeline may be used in the future in order to coregister the metal maps and the Spatial Transcriptomic data to the intact WSI.

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