The Effects of Phosphoprotein Enriched in Astrocytes-15 (PEA15) on Cerebral Endothelial and Neuronal Cell Density in Domestic Cats

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A loss-of-function mutation in phosphoprotein enriched in astrocytes-15 (PEA15) results in severe neurodevelopmental abnormalities in cats. Previous work done in collaboration with HudsonAlpha Institute of Biotechnology explored how PEA15 may be involved in normal neurodevelopment by examining brains in normal cats (unaffected) and those with the mutation (affected). A new RNAseq analysis technique, called cell-type deconvolution, was developed to determine changes in cellular subtype. The cell-type deconvolution study showed that transcripts specific to neuronal cells did not change, while copies of genes specific to endothelial cells were increased in affected compared to unaffected cats, which suggests that affected cats have either increased endothelial cell number or increased endothelial activity with little to no change in neuron cell number.

While these findings are promising, this new technique needs to be independently validated through methods such as immunohistochemistry (IHC). Additionally, a separate analysis, known as differential gene expression, indicated that affected cats have increased expression of genes associated with collagen production. Collagen is an important component of blood vessels and is closely associated with endothelial cells. Changes in cerebral endothelial cell number and function, as well as changes in collagen levels, are associated with various neurodevelopmental abnormalities. These findings may suggest a potential mechanism that contributes to the phenotype observed in cats with a loss of normal PEA15 function. Based on these previous findings, our objective was to (1) validate the cell-type deconvolution data through IHC staining and morphometric analysis, and (2) determine if there are changes in collagen thickness of cerebral vessels.

To address our first goal, sections of the cerebrum from affected (N=5) and unaffected (N=5) cats were independently stained for endothelial cell and neuronal cell detection, digitized, and analyzed using morphometric image analysis software to quantify the density of endothelial and neuronal cells in the samples. We generated multiple algorithms to analyze the same section of cerebrum in order to selectively identify the stain against the background. For endothelial cell detection, we successfully created two algorithms that accurately identified the stain. However, there was no significant difference ($p > 0.9999$ and $p = 0.3095$) between the two groups. This finding contrasted with what our cell-type deconvolution data suggested. Similarly, an algorithm was refined for neuronal cell detection. Consistent with the cell-type deconvolution data, we found that there was no significant difference ($p = 0.47$) between the two groups.

To address our second goal, sections of cerebrum from affected (N=4) and unaffected (N=5) cats were independently stained with Masson's trichrome to distinguish cells from surrounding collagen. Preliminary results suggest that there is an increase in collagen surrounding vessels of affected compared to unaffected cats. In future studies, we plan to measure the ratio of the thickness of the collagen compared to the thickness of the vessel wall for all samples and statistically evaluate difference in ratios between affected and unaffected cats.

In summary, cell-type deconvolution data were able to evaluate neuronal density with findings that were consistent with IHC; however, cell-type deconvolution data did not correlate with IHC staining for endothelial cells. This discrepancy in findings for endothelial cells may reflect a distinct change in endothelial cell activity rather than cell number. For example, endothelial cells can drive changes in collagen within vessels walls, which supports the finding of a subjective increase in collagen in the vessels of affected cats. However further study is needed to determine the significance of these findings.

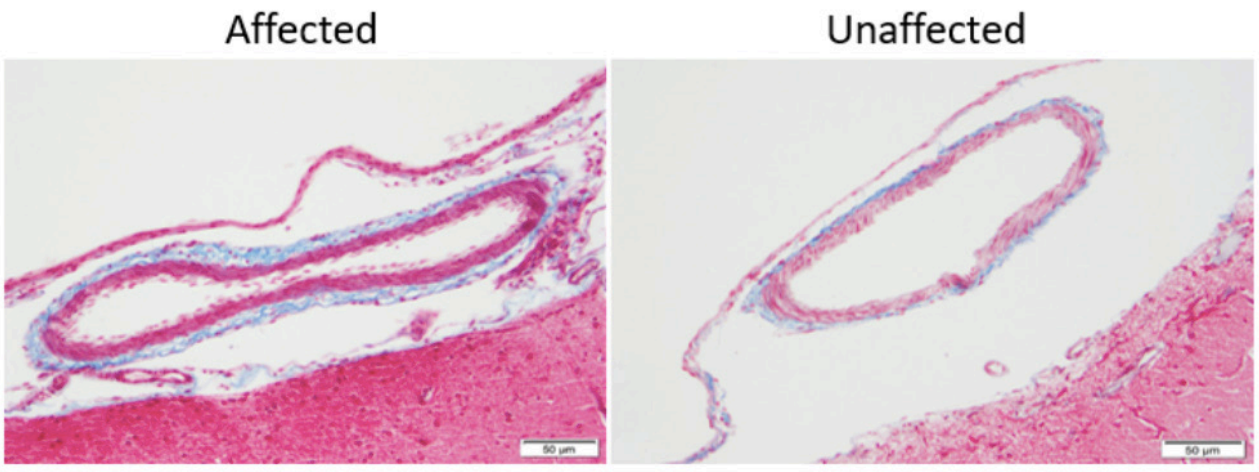

Figure 1. Masson's trichrome stain samples. Sections of cerebrum from affected (N=4) and unaffected (N=5) cats were independently stained with Masson's trichrome. These images represent blood vessels from unaffected cats (right) and affected cats (left). The blue around the blood vessel is collagen.

Statement of Research Advisor

Kacie Florus worked to develop and evaluate IHC stained slides of feline cerebral tissue in order to evaluate mechanisms that contribute to the serve phenotype observed in cats with a loss-of-function mutation in PEA15. Her work is an important contribution that has helped develop and validate new research tools in our lab.

– *Emily Graff, Pathobiology*