¹H NMR Lipidomic Analysis of Canine Stifle Joint Synovial Fluid to Detect Biomarkers of Medial Meniscal Injury

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Introduction

- Cranial Cruciate Ligament Disease/Rupture (CCLD/R)** is the primary degenerative disease afflicting the canine stifle joint ^{1,2,3}. The economic estimate of the impact of CCLD/R in 2003 was \$1.32 Billion ⁴.
- Meniscal Injury (MI) is a common sequelae of CCLD/R. It is reported that the medial meniscus is damaged in up to 77% of patients presenting with CCLD/R ^{5,6,7,8}, although true prevalence is thought to be closer to 60% ^{7,9}.
- The most represented type of meniscal injury is a longitudinal tear of the caudal pole/horn known as a bucket handle tear affecting the medial meniscus which accounts for around 57% of tears ^{5,6,7,8}.
- Nuclear Magnetic Resonance (NMR) spectroscopy can be used to investigate the metabolomic profile of complex mixtures, with the ability to quantify and qualify components of a mixture in a rapid and non-destructive fashion.

Objectives

“We hypothesise that the lipid profile of canine synovial fluid alters in the presence of meniscal injury.”

In order to identify potential differences, we elected to use ¹H Nuclear Magnetic Resonance (NMR) to detect differences in the spectra of synovial fluid, where there is both cranial cruciate ligament rupture and concurrent meniscal injury, to a control group with cranial cruciate ligament rupture only.

To the best of our knowledge, this is the first time the lipid profile of canine synovial fluid in response to meniscal injury has been interrogated.

Materials & Methods

Figure 1. Flow Diagram of authors controlled untargeted lipidomic approach of 18 stifle joint samples.

Results

Figure 2. **Top Left:** Authors observed spectra with highlighted peaks of interest. **Top Right:** PCA Plot from MetaboAnalyst with CCLD + MI in Green and CCLD only in Red. **Bottom Left:** Biplot from MetaboAnalyst showing drivers of variation. **Bottom Right:** Root Mean Square values of Biplot Scores.

PCA Plots of the samples showed little variation within the data set. Biplots determined **Unknown** peaks **17**, **7** and **8** as major drivers of variation within the experiment.

The spectra were compared to theoretical spectra obtained from the biological magnetic resonance databank. The spectra showed excellent correlation over the key peaks and as such **Unknown 17** was annotated as a **Cholesteryl ester** closely related to **Cholesteryl Palmitate** and **Unknown 7** and **8** are likely **Omega 6** and **Omega 9** Fatty Acids.

The authors intend to run internal validation using a range of pure samples of cholesteryl esters closely related to Cholesteryl Palmitate and the same nuclear magnetic resonance spectrometer to allow definitive identification of Peak 19.

Conclusion and Future Directions

This was a pilot study to determine the number of samples required in a larger experiment and determine proof of principle of the experimental workflow. In future studies, we will refine the extraction protocols utilized in this pilot study resulting in reduce distortion and noise within the spectra.

A much larger study population is needed. Additional internal validation for identification of potential lipids within the samples will facilitate molecular pathway analysis. Ultimately this will enable a greater understanding of how small metabolites present during meniscal injury contribute to the pathogenesis of the disease and if these could be diagnostic biomarkers.

References

Figures 1 & 2 generated with BioRender.Com

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