Metabolic Phenotypes Reflect Patient Sex, Age, BMI and Injury Status: A Cross-Sectional Analysis

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\textbf{References}

\textbf{Introduction:} Post-traumatic osteoarthritids (PTOA) is caused by knee injury\(^1\). Annual incidence rates of ACL injuries are 68.6 per 100,000 people with males having a slightly higher incidence rate compared to females\(^2\). Often, ACL injuries are accompanied by damage to other tissues and structures within the knee including the meniscus. Both injuries are known to cause PTOA, but underlyling cellular mechanisms driving the disease remain unknown. Injury, other risk factors that are associated with PTOA include sex, age, and BMI. Metabolomic profiling offers the potential to improve current understanding of the effects of injury on knee structures and tissues by identifying metabolic alterations that are affected, or induced, by different injury types. Additionally, using this approach allows patient-specific factors like sex, age, and BMI to be considered. Therefore, the goal of this study was to perform a cross-sectional analysis of synovial fluid (SF) metabolomic phenotypes comparing patient-specific factors including sex, age, BMI, and different knee injury pathologies.

\textbf{Methods:} In total, 33 knee arthroscopy patients participated in this cross-sectional pilot study under approval from the VCU IRB. Inclusion criteria were age between 18 and 65 years and no prior knee injuries before the current one (Table 1). De-identified patient information included sex, age, BMI, and injury pathology. Injury pathology groups included ligament (L), meniscal (M), and ligament and meniscal (LM). For statistics, patient data was divided into tertiles for age (18-29 years, 31-44, and 46-65) and BMI (18-24.9 kg/m\(^2\), 25-31.9, and 32-50). SF was obtained pre-procedure. Injury pathologies were assigned post-procedure based on the postop pathology report. For metabolomic analysis, SF metabolites were extracted using a methanol:aceton precipitation followed by 2 rounds of vortexing, centrifugation, and vacuum concentration. Samples were analyzed via liquid chromatography-mass spectrometry (LC-MS) (Waters UPLC Xevo and XCMS) and then log transformed and standardized. To statistically analyze samples and visualize metabolic differences between patient factors (i.e., sex, age, BMI) and injury pathology, MetaboAnalyst was used for principal component analysis (PCA), partial least squares-discriminant analysis (PLS-DA), volcano plots, fold change, and heatmap analysis. Following statistical analysis, the Mumichog algorithm was used to map clusters of key metabolites to biologically relevant pathways. Significance was determined using an FDR-corrected \textit{a priori} threshold of p < 0.05.

\textbf{Results:} To assess metabolic differences associated with patient-specific factors, PLS-DA revealed that the metabolomes of patients that differ by sex, age, and BMI are distinct from each other (Fig. 1A-C). Specifically, metabolites that differ in abundance between male and female patients included carnitine, pyruvate, tryptamine, and various glycan s. Considering age, metabolites highest in the youngest patients (18-29 y) were associated with terpenoid backbone synthesis, fatty acid biosynthesis, and steroid biosynthesis. Conversely, glycerophospholipid biosynthesis and amino acid metabolism (alanine, cysteine, and methionine) were highest in ages 31-44. Finally, N-acylglycan biosynthesis, porphyrin metabolism, and different amino acid metabolic pathways (lysine, valine, leucine, isoleucine) were highest in the oldest age category, 46-65. Similar to age, different metabolic pathways were associated with different BMI categories including glycerophospholipid and glycosphingolipid metabolism (highest BMI 32-50), tryptophan metabolism and primary bile acid biosynthesis (BMI 25-31.9) and folate biosynthesis (BMI 25-31.9, 32-50). Next, a median intensity heatmap analysis was performed to determine clusters of metabolites that were differentially regulated between different injury pathologies (Fig. 2). Metabolic pathways that exhibited the lowest alterations included xenobiotic metabolism, fatty acid oxidation, and dimethyl-branched-chain fatty acid mitochondria beta-oxidation (Fig. 2 C2). Conversely, aspartate and asparagine and glycerophospholipid metabolism were the highest in L injuries (Fig. 2 C3). Taken together, these results suggest that the SF metabolome of patients that differ by age, BMI, sex, and injury pathology are distinct from each other.

\textbf{Discussion:} The results of this cross-sectional pilot study suggest that patient-specific risk factors (i.e., age, BMI, sex) have distinct phenotypes. The detection of metabolites and generation of metabolomic phenotypes based on age, BMI, and sex can be used to improve patient treatment, identify patient-specific interventions, and allow for more precise medical treatment to benefit joint and patient health post-injury. Furthermore, this LC-MS based global approach provided clear differentiation between patients based on injury pathology. Previous studies have applied metabolomics to examine SF from patients with osteoarthritis and rheumatoid arthritis\(^3\), but no study to date has used this method to underpin metabolic shifts induced by different injuries. Importantly, we generated metabolic phenotypes and detected differences in pathway regulation within L, M, and LM injuries. Specifically, regulation of metabolites that correspond to fatty acid oxidation, dimethyl-branched-chain fatty acid mitochondria beta-oxidation, and amino acid metabolism differed between injury pathologies. Considering these phenotypic associations, a greater understanding of metabolic mechanisms associated with specific injuries and PTOA might yield data regarding how endogenous repair pathways differ between injury types. Furthermore, ongoing metabolomic analysis of SF in injured patients can be performed to monitor PTOA development and progression. Completion of this work may potentially lead to the identification of drug targets that slow, stop, or reverse PTOA progression considering injury type as well as patient-specific risk factors such as age, BMI, and sex. SIGNIFICANCE/Clinical RELEVANCE: The results of this study suggest that the SF metabolome is influenced by patient-specific factors and injury type. By employing metabolomics, a greater understanding of dysregulation occurring at the joint level post-injury may lead to an improved understanding of PTOA development as well as patient intervention and treatment.


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\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Synovial fluid-derived metabolite profiles differ based on patient (A) sex, (B) age, and (C) BMI.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{figure2.png}
\caption{Median intensity heatmap identifies clusters of metabolites that are differentially regulated between injury pathologies.}
\end{figure}

\begin{table}
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\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
\textbf{Sex} & \textbf{Age} & \textbf{BMI} \\
\hline
Female & 17 & 18-29 & 13 & 18-24.9 & 14 \\
Male & 16 & 31-44 & 10 & 25-31.9 & 13 \\
Total & 33 & 48-70 & 10 & 32-50 & 6 \\
\hline
\end{tabular}
\caption{Participant information.}
\end{table}