Phenotypes of osteoarthritis by global metabolomic profiling of human synovial fluid

Alyssa K. Carlson[1], Rachel A. Rawle[1], Cameron W. Wallace[1], Ellen G. Brooks[1], Erik Adams[1], Mark C. Greenwood[1], Merissa Olmer[2], Martin K. Lotz[2], Brian Bothner[1], and Ronald K. June[1]

[1]Montana State University, Bozeman, MT

[2]The Scripps Research Institute, La Jolla, CA

Introduction

- Osteoarthritis (OA) is a multifactorial disease with heterogeneous pathology
- OA has been described as having multiple phenotypes in which patients are classified by specific disease characteristics
- Global metabolomic profiling analyzes thousands of small molecule intermediates to generate a phenotype characterizing functional activity, and thus is a promising method for defining OA phenotypes

Hypothesis: Metabolic phenotypes exist between stages of OA (healthy, early, and late OA) and within stages of OA

How: Evaluate human synovial fluid (SF) using global metabolomic profiling by liquid chromatography-mass spectrometry (LC-MS)

Methods

1. 79 *post mortem* SF samples from Outerbridge-scored human knee joints were divided into three cohorts: healthy (grade 0; n=7), early OA (grades I-II; n=55), and late OA (grades III-IV; n=17)
2. Metabolites were extracted from SF using 50:50 water:acetonitrile solution
3. Samples were analyzed by LC-MS (Agilent 6538 Q-TOF MS in positive mode; resolution: 20,000ppm, accuracy: 5,000ppm)
4. Differences between cohorts and phenotypes were assessed using statistical analyses in MetaboAnalyst
5. Metabolite identity matches and enriched pathways were identified using MetaboAnalyst MS Peaks to Pathways

Results

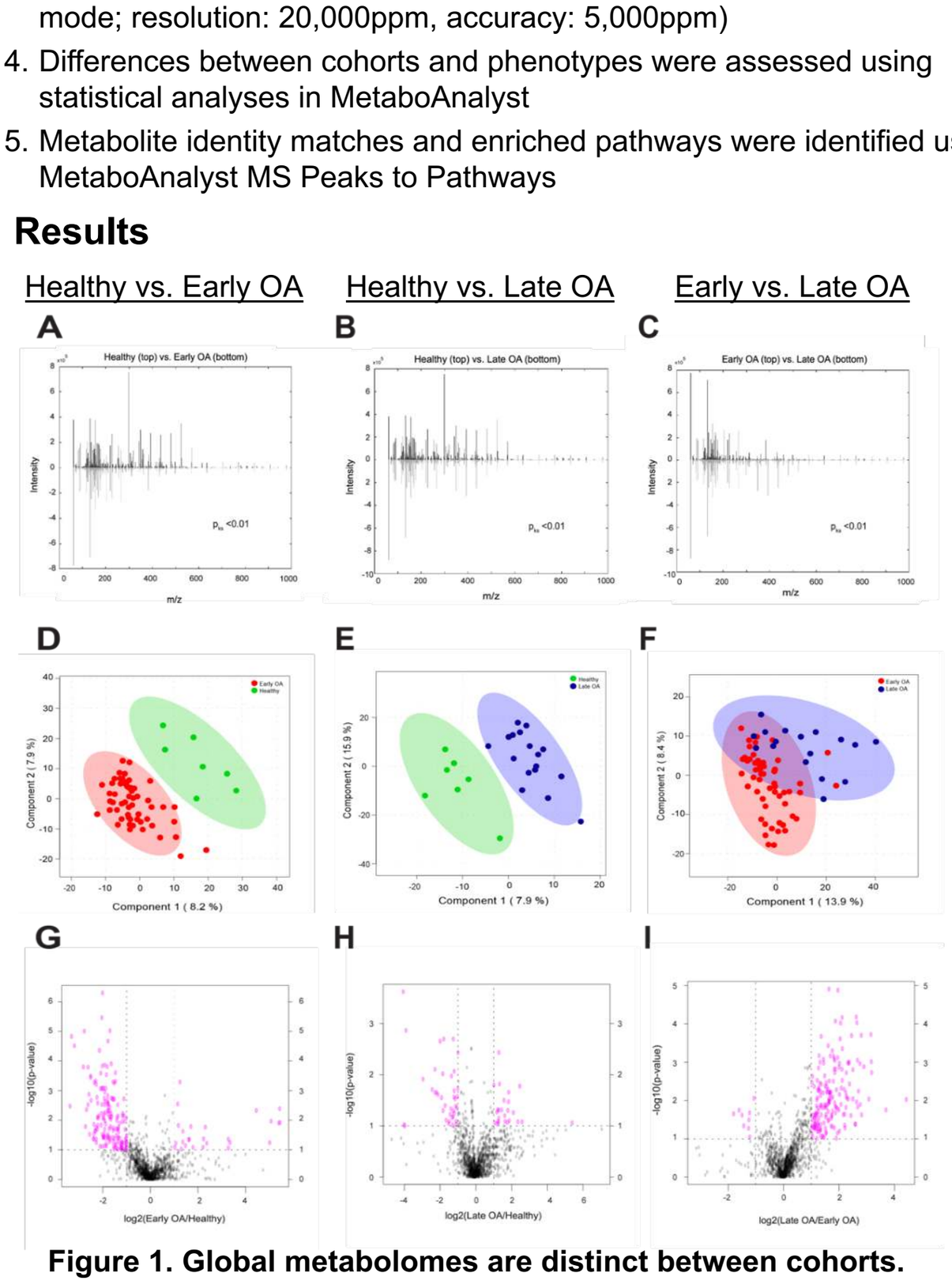

Figure 1. Global metabolomes are distinct between cohorts. **(A-C)** KS-tests comparing the median metabolite intensity distributions between groups revealed significantly (p_{ks}<0.01) different metabolomic profiles. **(D-F)** PLS-DA components 1 and 2 showed differences in metabolomic profiles of between groups, revealing clear separation between healthy and OA donors and some separation between early and late OA donors. **(G-I)** Volcano plot analysis between groups revealed metabolite features upregulated and downregulated by FDR-corrected p-value (p<0.05) and fold change analysis.

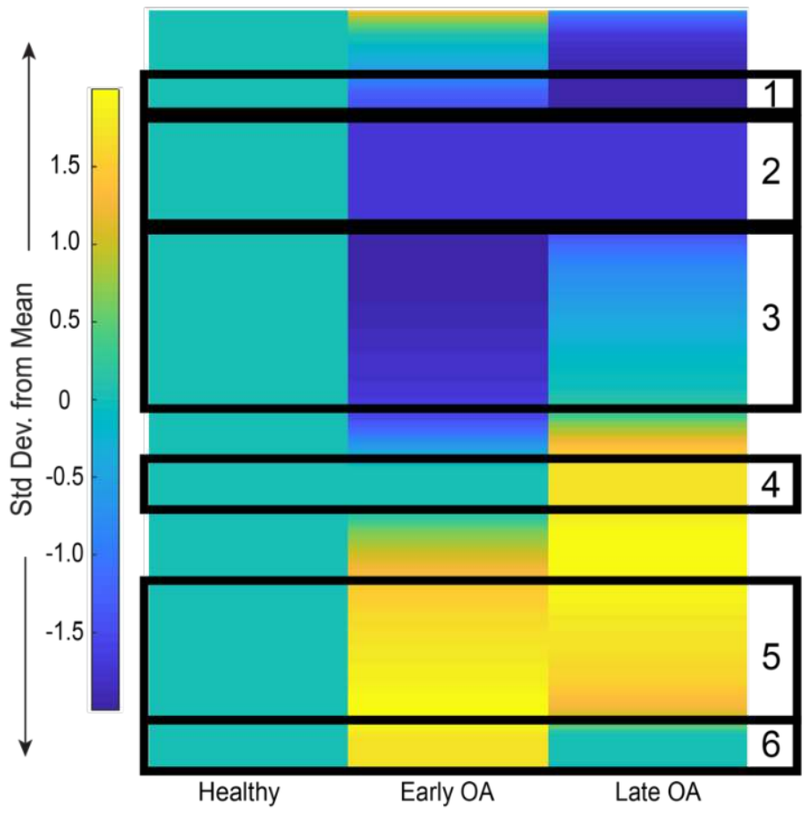

Figure 2. Metabolic changes in SF during early and late stage OA. Clustergram of median global metabolomic profiles of early and late OA SF. Data normalized to healthy SF. Selected clusters of co-regulated metabolite features are boxed in black and enriched for relevant pathways.

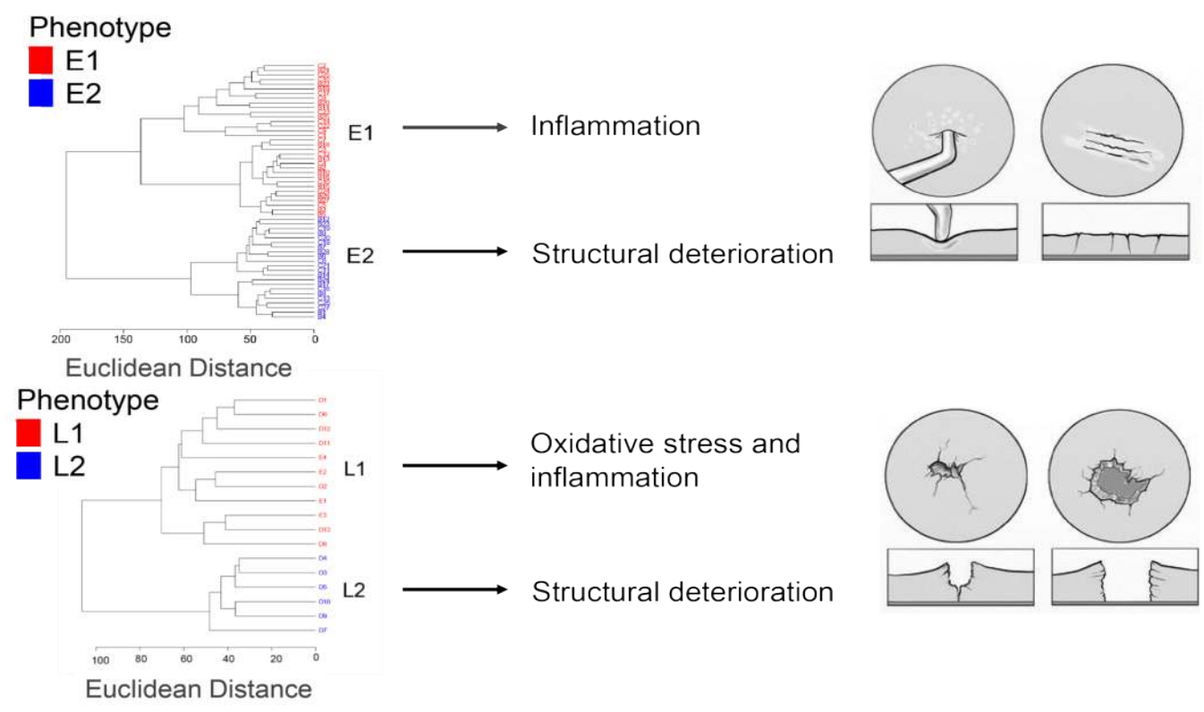

Figure 3. Metabolomic phenotypes of osteoarthritis. Cluster analysis revealed distinct metabolic phenotypes. Within Outerbridge grades I and II (early OA), metabolite features clustered into an E1 phenotype associated with inflammatory pathways and an E2 phenotype associated with structural degradation pathways. Within Outerbridge grades III and IV, metabolites clustered into an L1 phenotype associated with oxidative stress and inflammation and an L2 phenotype associated with structural degradation.

Conclusions

- LC-MS-based global metabolomic profiling of early and late OA SF showed distinct metabolic phenotypes within OA SF.
- OA SF exhibited evidence of altered extracellular matrix component metabolism, fatty acid and lipid metabolism, inflammation, central energy metabolism, oxidative stress, and vitamin metabolism.
- We identified two distinct phenotypes within both early OA and late OA. In both, phenotypes appear to have distinct biochemical pathways for either inflammatory or structural degradation processes.
- These results suggest that inflammation, oxidative stress, and structural deterioration may not be as closely linked in OA as previously thought.
- These findings support the heterogeneity of OA and the use of global metabolomic profiling as a means of classifying donors into additional phenotypes within disease stages.