Introduction

Rheumatoid arthritis, a debilitating, systemic inflammatory joint disease impacting 1-2% of the population, may be accompanied by alterations in specific metabolites. As an initial approach to investigating this possibility in a well-defined system we selected a murine model of rheumatoid arthritis, the KBxN mouse. In this transgenic model, a systemic inflammatory response is generated towards the ubiquitously expressed glucose-6-phosphate isomerase enzyme. Here we examine the metabolite profiles of KBxN mice and contextualize them with respect to rheumatoid arthritis mechanisms (Figure 1).

Methods

Sera from arthritic populations of KBxN mice that are genetically-predisposed to arthritis (N=15), as well as healthy parent strain population (N=22) were analyzed using ultrafiltration followed by 1H NMR spectroscopy.

A ‘Targeted Profiling’ approach [1] was used to identify and quantify 54 metabolites using Chenomx NMR Suite (Chenomx Inc, Edmonton Alberta). Metabolite identification was confirmed using 2D methods and sample spiking.

Subsequent multivariate analysis was performed using SIMCA-P+ (Umetrics, Sweden) to build an orthogonal partial-least squares discriminant analysis (OPLS-DA) model. The model was verified using a cross-validation approach, and a number of key metabolites identified as described below.

Results

Scores plot from multivariate statistical modelling using OPLS

Figure 4: Scores plot from the OPLS analysis of the metabolite concentrations obtained using the targeted profiling approach. A) Coloured according to type of mouse and B) coloured according to gender. Note that the first component in OPLS (x-axis) provides information on class separation, and as a result only the first component is considered for interpretation of metabolites relevant to arthritis. The first orthogonal component shows preliminary evidence of gender separation.

Summary of important metabolites

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Normal</th>
<th>Arthritis</th>
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<tbody>
<tr>
<td>Taurine</td>
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<tr>
<td>Glycine</td>
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<tr>
<td>Creatine</td>
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<td>Glutamate</td>
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<td>Hypoxanthine</td>
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<tr>
<td>1-Methylxanthine</td>
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<tr>
<td>3-Chorouracil</td>
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<tr>
<td>Methionine</td>
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<tr>
<td>Threonine</td>
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</tbody>
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Potential physiological role of metabolites

- Lipid metabolism
  - Glycerol, choline, acetylcholine, threonine
- Nucleic acid metabolism
  - Xanthine, hypoxanthine, uridine
- Reactive oxygen species (ROS) metabolism
  - Taurine, Glycine
- Methylation
  - Methionine
- Immune response in macrophages
  - Glycine (via N-glycine-gated chloride channel)

Conclusions

- Metabolite profiling of sera from mice models of human disease is a viable way to understand pathological mechanisms, and provide a means for evaluating the function of disease modifying drugs
- In the case of the KBxN mouse model, gender has little effect on an appropriately developed multivariate model
- Some of the metabolic changes are likely related to dietary considerations (e.g. lipid metabolism), although a number of specific inflammatory biomarkers are also evident
- Our results attest not only to the complexity of systemic inflammatory responses, but also the power of the experimental approach in being able to reveal such a wide variety of biomarkers.

Acknowledgements

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