43. ANALYSIS OF THE SYNOVIAL PROTEOME IN JUVENILE IDIOPATHIC ARTHRITIS

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Background: Juvenile idiopathic arthritis (JIA) comprises a heterogeneous group of chronic, childhood onset, autoimmune diseases with highly variable clinical presentations, outcomes and therapeutic responses. Although clinical decision making is often central and important method to pediatric rheumatologists of establishing likely diagnoses and outcomes, laboratory testing for pathogenic biomarkers within joint fluid and peripheral blood samples has been limited. In this paper we present preliminary data of the complex protein profiles within synovial fluid by 2-dimensional electrophoresis (2DE) methods.

Methods: 2DE is a powerful and sensitive technique for separating and analyzing protein mixtures from biological samples. Serial synovial fluid samples, collected from patients displaying a progression in disease, were clarified by centrifugation. Synovial fluid samples were prepared in sample rehydration buffer (8M Urea, 2% CHAPS, 0.5% (v/v) ZOOM carrier ampholytes (LKB) and 0.05% (v/v) bromophenol blue). The first dimension separation of proteins by isoelectric focusing based on isoelectric point or pl was carried out after applying 200 µg (by BCA protein assay) of synovial fluid to immobilized pH gradient (IPG) strips. The second dimension separation (based on molecular weight) was run using denaturing (4-12%) polyacrylamide gel electrophoresis (SDS-PAGE). The focused proteins were visualized on the completed 2DE gels by colloidal coomassie blue staining and digital images captured by a CCD camera with transillumination.

Results: The majority of focused protein spots were within pH range 4 – 7. Prominent disease-specific differences in protein expression patterns were observed between samples taken from the same knee in a patient displaying disease progression from mono- to pauci-articular JIA. Specific protein spot intensities from both high and low-mass regions of the gel were quantified using the Phoretics 2D software package (Non-linear Dynamics Ltd., UK). Protein spots with significant fold differences in expression were excised from gels, trypsin digested and further characterized by mass spectrometry.

Conclusions: The characterization of the synovial proteome with patients with JIA may reveal a small subset of biomarkers/putative therapeutic targets that play a specific role determining the pathophysiological state within the chronically inflamed joint. This large scale validation of differential protein expression profiles will facilitate the prediction of disease susceptibility, assist in diagnosis, further define disease staging, and could allow the selection of individualized therapies or monitoring of treatment responses.

44. TUMOUR NECROSIS FACTOR ALPHA (TNFα) SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs) IN THE IDIOPATHIC INFLAMMATORY MYOPATHIES (IIMs)

H. Chinoy1, F. Salway2, S. John2, W.E. Ollier2, R.G. Cooper1. 1Rheumatic Diseases Centre, Hope Hospital, Salford, United Kingdom; 2Centre for Integrated Genomic Medical Research, Manchester University, Manchester, United Kingdom

Background: To investigate the link between the IL-4, MCP1, IFNG gene polymorphisms and the IIMs

Methods: DNA samples were obtained from 286 Caucasian IIM patients, consisting 111 polymyositis (PM) (median age of onset 50.5 ±14.5 years), 98 dermatomyositis (DM) (49 ±13.3 years), and 77 polymyositis/overlap disease (PM/OL) (46 ±12.1 years), recruited via the Adult Onset Myositis Immunogenic Collaboration (AOMIC). All had definite myositis, as per the Tantimon et al criteria. The polymorphic positions of 15 SNPs and 2 microsatellite markers were typed using a fluorescence-based primer method (SNAPShot™). The chi-squared test was used to test for associations (data expressed as odds ratios and 95% confidence intervals) and compared to 167 ethnically matched controls. Genotype frequencies for each SNP was tested for Hardy-Weinberg equilibrium in the control population. Linkage disequilibrium (LD) between SNPs was calculated using r values. A haplotype trend regression analysis was also performed. This uses the Expectation-Maximization (EM) algorithm to estimate haplotype frequencies, and then relates haplotype from microsatellite polymorphism to disease outcome, using a regression-based approach.

Results: Three SNPs (IL-4-4, IFNG-5, MCP1-5) were found to be monomorphic and omitted from the study. There was very strong linkage disequilibrium present within the 3 SNP groups. No significant risk factors were demonstrated for any IIM subgroup, or when the subgroups were combined. Haplotype trend regression analysis also failed to demonstrate any significant associations. An increased number of IL-4-5 homozygotes were observed in DM (69% patients vs 58% controls, p=0.07). Haplotype frequency analysis showed a reduction in the combination 1C/2T/4A in disease vs controls (controls 21.1%, PM 14.5%, DM 16.9%, PM/OL 9.2%).

Conclusions: The SNPs IL-4, MCP1 and IFNG show no significant associations in the IIMs, subgroup or when combined. We conclude that the degree of expression of these cytokines in IIM inflammatory infiltrates is not genetically determined.

45. INTERLEUKIN-4 (IL4), MONOCYTE CHEMOATTRACTANT PROTEIN-1 (MCP1) AND INTERFERON-GAMMA (IFNG) SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs) IN THE IDIOPATHIC INFLAMMATORY MYOPATHIES (IIMs)

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46. DEVELOPMENT OF HUMAN ANTIBODY FRAGMENTS AGAINST COLLAGEN TYPE II AUTOANTIGENS IN RHEUMATOID ARTHRITIS, USING PHAGE DISPLAY LIBRARIES

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Background: Rheumatoid arthritis (RA) is an autoimmune disease which is characterised by chronic inflammation of the joints, associated with synovitis and erosion of the cartilage and bone. The destruction is due to the action of pro-inflammatory cytokines, free radicals and matrix metalloproteinases (MMPs). A new generation of drugs, infliximab, a chimeric humanized anti-TNF antibody, is claimed to improve disease activity in RA.

Methods: We used two semi-synthetic phage display libraries1,2 to isolate antibodies against type II collagen (CII), which is the major component of human articular cartilage and a characterised autoantigen in RA. We used semi-synthetic phage display libraries1,2 to isolate antibodies against type II collagen (CII), which is the major component of human articular cartilage and a characterised autoantigen in RA.

Results: Uncharged polar amino acids (N, S, T or Y) were found to be very frequent in both VH-CDR3 and VL-CDR3. None of the clones selected in this work reacted against human IgG (hu-IgG). This was despite striking se-
Gout is characterised by tissue deposition of monosodium urate crystals. The various US features described in gout include: (1) mutton-fat calcifications (< 2 mm thick) (35%); (2) tophus (hard or soft) (74%); (3) mutton-fat calcifications (31%); (4) power Doppler signal (79%); (5) joint effusion (87%).

Pathological findings Number of patients (%)

- Joint effusion -34 (87)
- Synovial hypertherpy -30 (95)
- Power Doppler signal -31 (79)
- Tophus (hard or soft) -28 (74)
- Double contour sign -12 (31)

Conclusions: These pathological findings may be used to predict patients at risk of developing tophi. Further studies are needed to address this issue.

Metabolic and crystal arthropathies

47. SONOGRAPHIC FEATURES OF GOUT: A PICTORIAL ESSAY

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Background: Gout is characterised by tissue deposition of monosodium urate crystals as a result of hyperuricaemia that can result in acute attacks of tophaceous gout. The aim of this pictorial essay is to show the potential value of high-resolution ultrasonography (HRUS) in the depiction of bone and soft tissue abnormalities induced by MSU crystal deposition at 1st MTPJs in patients with gout.

Methods: HRUS examinations were performed using a Siemens Sonoline Antares with 13-5 linear array transducer. The medial and dorsal aspect of the 1st MTPJs were examined in each patient. Sonographic findings were visualised on two perpendicular scanning plains. The pictures presented in this pictorial essay, were selected from those obtained from a total of 39 patients with gout.

Results: HRUS allowed the depiction of several pathological findings (Table 1).

- In patients with an acute attack of gout 1st MTPJs showed anechoic joint cavity widening and marked edema of surrounding soft tissues. In patients with tophaceous gout HRUS appearance of tophi depends on the density of the MSU crystal deposits. Hard consistency (hard tophus) appeared as a hypoechogenic band (corresponding to the outer border of the urate deposit) with a posterior acoustic shadow. Soft consistency (soft tophus) showed an inhomogeneous echotexture. In 12 (31%) patients with chronic gout, US detected an evident thickening of the cartilage superficial margin, which appeared as thick as the deeper margin (double contour sign). The erosive involvement was mainly multifocal (26 out of 32 patients with HRUS evidence of bone erosion) and located on the medial aspect of the metatarsal head (in 31 patients).

Table 1. Pathological findings in either 1st MTPJ depicted by HRUS.

<table>
<thead>
<tr>
<th>Pathological finding</th>
<th>Number of patients (%)</th>
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<tbody>
<tr>
<td>Joint effusion</td>
<td>-34 (87)</td>
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<tr>
<td>Power Doppler signal</td>
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</tr>
<tr>
<td>Tophus (hard or soft)</td>
<td>-28 (74)</td>
</tr>
<tr>
<td>Bone erosion</td>
<td>-32 (82)</td>
</tr>
<tr>
<td>Double contour sign</td>
<td>-12 (31)</td>
</tr>
</tbody>
</table>

Conclusions: A wide range of pathological changes can be depicted by HRUS at 1st MTPJs of patients with gout. A relevant finding was the high number of MTPJs showing US evidence of bone erosions, especially on the medial aspect of the metatarsal head. Although some of these sonographic features are not specific, some others could be of diagnostic value. Further studies are needed to address this issue.