THU0023 SYNOVIAL FLUID MIRNAS MULTIMARKER ANALYSIS IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: Recent epigenetic studies reveal the pathogenic role of micro-ribonucleic acids (microRNAs) and their targets in the inflammatory process in rheumatoid arthritis (RA). miRNAs play crucial role in controlling and modulating immunity and their abnormal expression has been linked to the deregulated function of regulatory T cells, to the chronic synovial inflammation and bone destruction[1–3].
Objectives: To perform a multimarker analysis of synovial fluid (SF) expression levels of miR-146a, miR-155 and miR-223 in RA patients in regard to their role as diagnostic biomarkers.
Methods: Total RNA was isolated from the SF of 48 RA patients and 11 healthy controls (HCs) and expression levels of miR-146a, miR-155 and miR-223 were determined by quantitative real-time polymerase chain reaction (qPCR), SybrGreen technology. Relative changes of gene expression levels of the miRNAs were calculated by $2^{-\Delta\Delta Ct}$method and SPSS were used for statistical analysis. RNU6B gene was used as a reference control for normalization. Receiver operating characteristic (ROC) curve analysis using RQ values was constructed in order to evaluate the diagnostic accuracy of these miRNAs in SF for distinguishing RA patients from HCs.
Results: miR-146a, miR-155 and miR-223 showed overexpression in RA SF compared to HCs (in 70.83%, 79.17% and 79.17% of the patients, respectively) and could be used to differentiate RA patients from HCs ($p=4.8x10^{-4}$, $p=8x10^{-5}$ and $p=2.8x10^{-4}$, respectively). The ROC curve analysis showed diagnostic accuracy for miR-146a with area under the curve (AUC)=0.769, 95% CI=0.600÷0.938, p=0.006, with sensitivity of 75% and specificity of 72.3%; AUC for miR-155 was 0.858 (95% CI=0.757÷0.959, $p=2.3x10^{-4}$) with sensitivity of 81.3% and specificity of 81.8%; AUC for miR-223=0.841 (95% CI=0.724÷0.958, $p=4.6x10^{-4}$), with sensitivity of 87.5% and specificity of 72.7%. The diagnostic accuracy improved when performing a multimarker analysis with AUC for the combination of miR-146a and miR-155=0.871 (0.776–0.967, $p=1.4x10^{-4}$), AUC for miR-146a and miR-223=0.867 (0.753–0.982, $p=1.6x10^{-4}$), AUC for miR-155 and miR-223=0.907 (0.823–0.991, $p=2.6x10^{-5}$). AUC for the combination of all three miRNAs was 0.915 (0.840–0.990, $p=2.02x10^{-5}$) with 89.6% sensitivity and 81.8% specificity.
Conclusions: Local miRNA expression levels could serve as diagnostic biomarkers in RA patients. The multimarker analysis of the expression levels of miR-146a, miR-155 and miR-223 in SF has better diagnostic accuracy than their single use in the clinical practice.
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Acknowledgements: The study was supported by Grant 14-D/2012 and 55/2013 funded by MU-Sofia.
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.6406

THU0024 WHOLE GENOME LINKAGE AND EXOME SEQUENCING ANALYSES IN AN AUTOSOMAL RECESSIVE TAKAYASU ARTERITIS FAMILY

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Background: Takayasu arteritis (TA) is a rare chronic inflammatory disease of the aorta and its major branches, seen predominantly in females. Its etiology is unknown, however, there is a growing body of evidence to suggest genetic contribution to the pathogenesis of the disease: a) The disease is relatively frequent in Asia, b) Several familial cases of TA have been published (1) and even, autosomal recessive inheritance pattern has been suggested (2), c) Genetic association with HLA-B*52 across multiple ethnicities has been confirmed (3), and d) A multi-ethnic genome-wide association studies (GWAS) study in TA established additional genetic susceptibility loci (4–5).
Objectives: We studied a consanguineous family consisting of two affected and one unaffected sibs and their healthy parents in order to identify the causative mutation or linked loci.
Methods: Whole genome single nucleotide polymorphism (SNP) genotyping was performed for five family members using Illumina OmniExpress-24 BeadChip targeting ~700,000 SNP markers. Using genotyping data, we performed multi-point parametric linkage analysis assuming recessive inheritance and complete penetrance. Also, exome sequencing was performed for index patient to search for a rare, homozygous deleterious variant in the possibly linking regions.
Results: Whole-genome linkage analysis resulted in 25 genomic regions with LOD score above 1.50. Within the family members, all candidate regions shared homozygosity by only affected individuals. Causative variant search in linkage regions identified seven homozygous candidate variants in which five of them were located in 19q13.33. Candidate non-synonymous variants were found in *ANXA8L*, *EHBP1L1*, *MYH14*, *KCNJ14*, *SYNGR4*, *TULP2* and *SHANK1* genes.
Conclusions: This is the first whole genome linkage analysis in a TA family with recessive inheritance. Linkage and following exome sequencing analyses revealed seven possible variants that may be causative to disease. Further variant and candidate gene investigations are still in process.
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Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.6370

THURSDAY, 15 JUNE 2017

Cytokines and inflammatory mediators

THU0025 IL-6 TRANS-SIGNALING CAUSES ACCELERATED ATHEROSCLEROSIS IN DISEASE PRONE ANIMALS

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Background: Cardiovascular (CV) disease is a major cause of mortality in patients with rheumatoid arthritis (RA). CV risk is increased early in the disease course. Subclinical inflammation and dyslipidaemia are often seen in RA before patients become symptomatic, suggesting the presence of subclinical CV disease. Inflammation, as measured by acute phase reactants, is associated with CV disease in RA. Interleukin (IL)-6 is a major driver of the acute phase response in RA. Notably, systemic elevations in inflammatory cytokines including IL-6 correlate with CV risk. Importantly, IL-6 regulates both immune homeostasis and inflammatory processes linked with chronic disease progression. Control of these processes is regulated by two modes of IL-6 signaling; classical IL-6 receptor signaling and IL-6 trans-signaling. Cellular responses controlled by IL-6 trans-signaling are mediated via soluble IL-6 receptor (sIL-6R) and is widely considered to promote deleterious pro-inflammatory outcomes [1]. We hypothesize that atherosclerosis may predate diagnosis of RA, and is accelerated by IL-6 trans-signaling during active arthritis. Here, we investigate this hypothesis using the established ApoE-deficient (*apoE*$^{-/-}$) mouse model of atherosclerosis.
Objectives: To examine the effect of IL-6 classical and trans-signaling on atherosclerosis by administering IL-6 or Hyper-IL-6 (a IL-6: sIL-6R fusion protein) to *apoE*$^{-/-}$ mice.
Methods: Male *apoE*$^{-/-}$ mice were fed a high-fat diet for 8 weeks starting at 8 weeks of age. Mice were divided into 4 groups. Group 1 received IL-6 (160 ng twice weekly, delivered i.p.), and Group 2 and 3 received Hyper-IL-6 (500 ng and 1 μg delivered i.p. twice weekly) for 8 weeks. Group 4 received PBS twice weekly for 8 weeks. Serial transverse 7 um brachiocephalic artery cross-sections were cut and stained with haematoxylin and oil red-O. Lesion size was determined by computer-assisted morphometry, using Image J on stained sections. Brachiocephalic plaque size in mice treated with PBS, IL-6 and Hyper-IL6 were compared using ANOVA and post-hoc Tukey t test.
Results: Mice treated with Hyper-IL-6 1μg had significantly larger brachiocephalic plaques (mean plaque area 0.73±0.04 mm^2) than those administered PBS (0.018±0.01 mm^2, p <0.001), and IL-6 (0.033±0.017 mm^2, p =0.015; Fig.1A). Similarly, mice administered Hyper-IL-6 [1μg] had a significantly higher percentage of the brachiocephalic artery occupied by plaques (45.3±18.1%) compared to those administered PBS (10.38±6.7%, p <0.001) or IL-6 (20.1±10.2%, p =0.002) (Fig.1B). Mice administered Hyper-IL-6 [0.5μg] had significantly higher percentage plaque (27.7±16.2%) than PBS administered mice, p=0.015. There was no significant difference in total cholesterol, HDL, LDL, triglycerides, free fatty acids or cholesterol:HDL ratio between the groups.

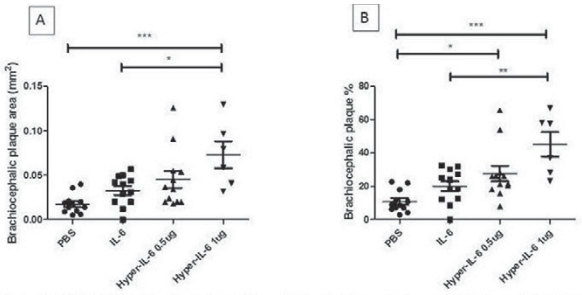

Figure1. (A) Significantly larger brachiocephalic plaque area in *apoE*$^{-/-}$ mice administered Hyper-IL-6 1μg compared to mice administered IL-6 or PBS. B) Significantly higher brachiocephalic plaque percentage in *apoE*$^{-/-}$ mice administered Hyper-IL-6 compared to PBS, and significantly higher brachiocephalic plaque percentage in *apoE*$^{-/-}$ mice administered hyper-IL-6 1μg compared to IL-6.

Conclusions: IL-6 trans-signaling leads to accelerated atherosclerosis in disease susceptible animals. This effect is independent of changes in serum lipid profiles.

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Acknowledgements: This work was funded by Arthritis Research UK, grant number 20760.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.2871

THU0026 OSM IS MORE EFFECTIVE THAN IL-6 AT INDUCING ENDOMT OF HUMAN DERMAL MICROVASCULAR CELLS

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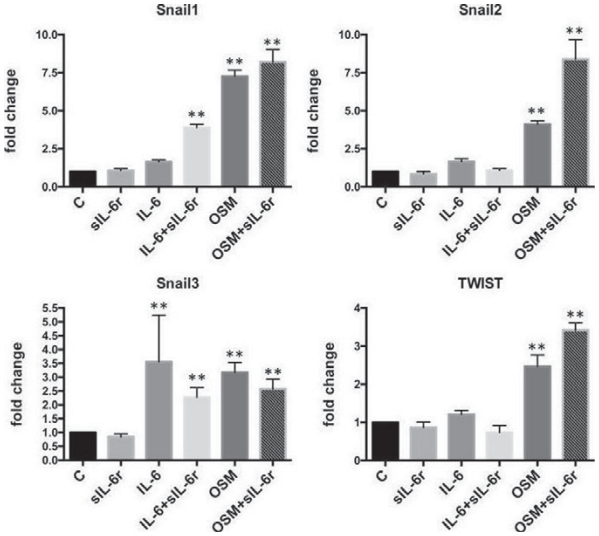
Background: Oncostatin-M (OSM) and interleukin-6 (IL-6) are members of the IL-6 superfamily and signal via glycoprotein 130 (gp130). OSM signals with either the type I receptor complex, gp130/ Leukemia Inhibitory Factor Receptor (LIFR), or the type II receptor complex, gp130/OSM receptor (OSMR), whilst IL-6 signals via gp130 with the IL-6 receptor (IL-6R) or by trans signalling with soluble IL-6R (sIL6-R). Endothelial cells (ECs) express both gp130 and OSMR [Brown TJ et al 1991], however it is unclear whether ECs express IL-6R [Romano M et al 1997; Nilsson MB et al 2005]. Endothelial to mesenchymal transition (EndoMT) is the phenotypic transition of ECs into mesenchymal cells where ECs lose their specific EC markers, detach from the endothelial layer and initiate the expression of mesenchymal cell products. EndoMT is associated with vascular dysfunction, one of the early manifestations of systemic sclerosis. The role of OSM and IL-6 in EndoMT has not yet been fully elucidated.

Objectives: To determine the effect of OSM and IL-6/sIL-6R on microvascular EC migration, proliferation and EndoMT.

Methods: Human dermal microvascular ECs (HDMECs) were treated with human recombinant proteins OSM (1–100 ng/mL), IL-6 (10–100 ng/mL) and sIL-6R (10–100 ng/mL). Cell migration and proliferation were measured with Live-Cell imaging system over 50 hours and analysed using a two-way ANOVA. Secretion of Collagen type I protein was measured at 48 hours by western blot analysis of media supernatant from HDMEC cultures. Changes in VE-Cadherin and F-actin expression were examined by immunofluorescence over 72 hours.



Gene expression was measured at 3 hours using quantitative RT-PCR analysis and analysed by Student's paired T-Test.

Results: OSM and IL-6, with or without IL-6R, significantly increased ($P<0.001$, n=3 donors) HDMEC migration and proliferation and secretion of extracellular matrix (ECM) protein Collagen I compared to the control group. OSM, but not IL-6 with or without sIL-6R, reduced expression of EC marker VE-Cadherin and increased expression of elongated F-actin stress fibres (n=2 donors). OSM significantly affected ($P<0.05$, n=3 donors) expression of EndoMT genes *SNAIL1*, *SNAIL2*, *SNAIL3* and *TWIST* [Figure 1] and ECM genes *MMP1*, *MMP2 TIMP1* and *TIMP2* compared to the control. Many of the gene changes in response to OSM were further augmented by co-stimulation with sIL-6R. IL-6 with or without sIL-6R only significantly affected ($P<0.05$, n=3 donors) EndoMT genes *SNAIL1* and *SNAIL3* and ECM gene *TIMP1*.

Conclusions: OSM induced a stronger EndoMT phenotype in HDMECs in comparison to IL-6, suggesting that OSM is capable of initiating EndoMT activity in microvascular cells. The augmented effects observed for OSM with sIL-6R also suggests that OSM is capable of binding the sIL-6R and initiating signalling in HDMECs *in vitro*.

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Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.1271

THU0027 CLUSTERIN IS ELEVATED IN SERUM AND MUSCLE TISSUE IN IDIOPATHIC INFLAMMATORY MYOPATHIES AND IS ASSOCIATED WITH DISEASE ACTIVITY

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Background: Clusterin (also known as apolipoprotein J) is a molecular chaperone that participates in inflammatory and apoptotic processes. Recent data indicate its possible protective role in the development of chronic autoimmune disorders.

Objectives: The aim of this study was to analyse the skeletal muscle expression of clusterin and its serum levels in patients with idiopathic inflammatory myopathies (IIM) and in healthy donors, and to examine the association of clusterin with disease activity.

Methods: Clusterin mRNA expression in skeletal muscle specimens, obtained by muscle biopsy (mini-invasive Bergstrom technique), was determined using qPCR in 10 patients with IIM and 10 healthy subjects. Serum concentrations of clusterin were measured by ELISA (Biovendor) in 65 patients with IIM (27 dermatomyositis (DM), 28 polymyositis (PM), 10 immune-mediated necrotizing myopathy (IMNM)) and in 54 healthy individuals. Disease activity was assessed using myositis intention to treat index (MITAX), myositis disease activity assessment visual analogue scales (MYOACT), health assessment questionnaire (HAQ) and global disease assessment evaluated by doctor and patient. Data are presented as mean ± SD.

Results: Clusterin mRNA expression in skeletal muscles was increased in patients with IIM compared to healthy donors (p=0.029). In addition, serum clusterin levels were significantly higher in all IIM patients than in healthy subjects (87.1±22.8 vs 68.4±12.4, $p<0.0001$) and also in individual subsets of patients in comparison to the control group (DM: 87.7±24.7, PM: 86.1±23.2, IMNM: 88.15±18.0, $p<0.0001$ for all). Clusterin levels in all patients with IIM positively correlated with MYOACT (r=0.337, p=0.008), MITAX (r=0.357, p=0.004) and global disease assessment evaluated by doctor (r=0.309, p=0.015). In patients with DM, positive correlations with MYOACT (r=0.499, p=0.009), MITAX (r=0.491, p=0.009), HAQ (r=0.470, p=0.014), global disease assessment evaluated by doctor (r=0.559, p=0.004), γ-glutamyl transpeptidase and asparate aminotransferase were found (r=0.504, p=0.007; r=0.429, p=0.025, respectively). PM and IMNM subsets showed no significant association.

Conclusions: We demonstrate increased local and systemic expression of clusterin in IIM patients compared to healthy individuals and its association with disease activity, especially in dermatomyositis.

Acknowledgements: Supported by the project of MHCR for conceptual development of research organization 00023728 and project NV16–33746A.

Disclosure of Interest: None declared

DOI: 10.1136/annrheumdis-2017-eular.5792