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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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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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.