Conclusions: Activation of ER α significantly enhances the miR processing, and affects the profile of miR transcription in female RA patients. The change in miR profile during E2-treatment could contribute to a significantly change in the miR landscape and disposition of intracellular processes in RA.

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THU0016

TRANSMITOCHONDRIAL CYBRIDS SHOW THAT OXPHOS VIA, BUT NO GLYCOLYSIS VIA, IS INVOLVED IN THE ATP REDUCTION OF OA HUMAN CHONDROCYTES

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Background: Mitochondrial dysfunction is well documented in OA and has the capacity to alter chondrocyte function and viability, contributing to cartilage degeneration. It is important to evaluate the influence of mitochondria in the pathogenesis of OA using an *in vitro* model to explain the functional consequences of this association and help us to identify potential diagnostic biomarkers and/or therapeutic targets. Transmitochondrial cybrids are a useful cellular model to study the mitochondrial biology and function implications in the cellular behaviour, since they carry different mitochondrial variants with the same nuclear background, therefore, excluding the variations because of nuclear genome.

Objectives: The aim of this work is test mitochondrial activity in the OA chondrocytes using transmitochondrial cybrids with mtDNA from healthy donors (without OA) and from patients with OA.

Methods: Cybrids were developed using 143B. TK⁺ Rho-0 cell line (nuclear donor) and platelets (mitochondrial donors) from healthy and OA donors. Human articular chondrocytes were obtained from patients with hip replacement. The mtDNA copy number was measured by real-time PCR method. The ROS production was evaluated using flow cytometry. The metabolic status was evaluated by glucose consumption and glucose oxidation. The glycolytic activity was measure after addition of glucose, oligomycin and 2-dioxylglucose using Seahorse XFp (ECAR). The OXPHOS function was evaluated by SeaHorse XFp (OCR) after addition of oligomycin, FCCP and Rotenone/Antimycin. Appropriate statistical analyses were performed with GraphPad Prism v6.

Results: The analysis of mtDNA copy number showed that the OA have higher levels than N in cybrids and human chondrocytes. The analysis of ROS production showed that OA had higher levels than N in both types of cells. The metabolic status analysing glucose consumption, glucose oxidation and total glucose cellular uptake reflected higher values in OA cybrids than N cybrids. But the analysis of glycolysis data showed lower values in OA than N cybrids. The analysis of ATP obtained through glycolysis did not show any difference between cybrids. The analysis of OXPHOS function showed that OA had lower basal respiration and maximal respiratory capacity than N in both types of cells. The ATP obtained via OXPHOS was lower in OA than in N.

Conclusions: The analysis of OXPHOS function supports the participation of mitochondria in cybrids and human chondrocytes metabolism. Both types of cells use the mitochondria to obtain ATP and OXPHOS via, but no glycolysis, is involved in the reduction of ATP synthesis by OA cells. All these data support that N cybrids and chondrocytes use mitochondria with more efficiency.

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THU0017

DNA METHYLATION OF REGULATORY SITES OF HAND OSTEOARTHRITIS SUSCEPTIBILITY GENES IN FINNISH WOMEN

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Background: Despite the hard effort in OA genetic studies only a small part of the estimated effect has been found so far and thus the focus has been changing from genetic to epigenetic studies. The most widely studied epigenetic control mechanism is DNA methylation. There are only few studies on hand OA concerning DNA methylation but their results are promising.

Objectives: Our aim was to study methylation of the regulatory sites of our previously found hand OA susceptibility genes and to replicate some previously reported methylation sites associated with hand OA, in our Finnish women hand OA material.

Methods: The study design was cross-sectional. Bilateral hand radiographs of 542 occupationally active Finnish female dentists and teachers aged 45 to 63 and

classified them according to the presence of OA by using reference images. Radiographic hand OA at least mild, in at least three joints (ROA2_3), and radiographic findings summary score (OASUM) were used to define hand OA status. The methylation percentages were determined by bi-sulfite converted DNA pyrosequencing with commercial CpG Assays. Statistical analyses were performed using hierarchical multiple linear regression.

Results: Of the studied methylation sites the COL2A1_1 methylation site was associated with ROA2_3 (p=0.04). Also ALDH1A2_01 methylation site was associated with OASUM (p=0.02). When the data was stratified by occupation the association was only significant, and stronger, in teachers but not in dentists (COL2A1_1 p=0.02 vs. p=0.36 for ROA2_3, and ALDH1A2_1 p=0.01 vs. p=0.36 for OASUM, respectively). The studied methylation sites in TGFB1, RRP9 and TRAPP5 genes had methylation percentages under the detection limit and they were excluded from the analysis.

Conclusions: Our results lend further support to COL2A1 and ALDH1A2 being hand OA susceptibility genes at the epigenetic level.

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THU0018

MIRNAS-146A AND -499 GENE EXPRESSION AND THEIR POLYMORPHISMS AS DIAGNOSTIC MARKERS FOR RHEUMATOID ARTHRITIS

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Objectives: To investigate the expression of miRNAs-146a and -499 and their polymorphisms in Egyptian patients with RA and to evaluate their relationship to clinical manifestations and disease activity.

Methods: Fifty-two RA patients and 56 matched controls were studied. Disease activity score-28 was assessed. MicroRNAs expression and polymorphisms were assayed by polymerase chain reaction (PCR).

Results: Patients mean age was 39.5±10.8 years and the disease duration of 7±5.1 years. The DAS28 was 3.1±1.7; 23 were in remission, 5 had mild disease activity, 18 moderate and 6 severe. There was a 15.5±27.2 fold increase in miRNA-146a and 3.3±6.1 in miR-499. The fold change of miRNA-146a was significantly higher in those without joint deformities (n=18) (28.1±42.6) compared to those with (8.8±8.4; p=0.01). Both miRNA-146a and -499 fold change were significantly decreased in those with a positive ANA (n=7) (2.5±2.1 and 0.07±0.08) compared to those with a negative test (18.1±29.6 and 3.8±6.5; p=0.002 and p=0.001 respectively). The fold changes tended to be higher in those in remission compared to active patients. However, miRNA-499 in those with severe disease activity tended to be higher. There was no significant correlation of the fold change of the miRNAs with the clinical manifestations or medications received. Only ANA positivity significantly inversely correlated with the fold increase in miRNA-146a (r=-0.42, p=0.003). The fold change in miRNA-146a significantly correlated with miRNA-499 (r=0.56, p<0.0001).

Conclusions: Both miRNAs-146a and -499 are highly expressed in RA patients and can be considered as diagnostic markers. Increased expression of miRNA-146a expression is protective in those with negative ANA and both in those without joint deformities.

Disclosure of Interest: None declared

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THU0019

ARE MICRORNAS A MOLECULAR CLOCK? THE NEWCASTLE THOUSAND FAMILIES COHORT STUDY

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Background: No single biomarker has been identified for monitoring ageing trajectory. To date, biological clocks are based on DNA methylation, telomere length, p16^{ink4a} expression and microsatellite mutations¹. Body ageing is a complex phenomenon, including a progressive, pro-inflammatory state, termed 'inflammaging'. MicroRNAs have been linked with cellular senescence and inflammaging. MicroRNAs are short, non-coding sequences of RNA regulating post-transcriptional gene expression with impressive stability and ubiquitous presence, making them ideal candidate biomarkers. Previous studies of circulating microRNAs in ageing were small-scale² or compared individuals of different ages, which is methodologically less robust³. MicroRNA biomarkers can bring greater understanding to

ageing mechanisms, diagnosis/prognosis of age-related disease and even novel treatment targets.

Objectives: To quantify relationships between circulating microRNA expression and biological ageing and determine whether microRNAs may be a molecular clock.

Methods: This pilot work is a nested substudy within a prospective, longitudinal birth cohort from May/June 1947 (the Newcastle Thousand Families Study NTFS). Serum samples from 23 subjects taken at ages 50 and 62 years were extracted from the biobank. HTG EdgeSeq microRNA whole transcriptome assay was performed, measuring expression of 2083 human microRNA transcripts using an array followed by next generation sequencing. Global microRNA expression profiles were generated and analysed using this technology, profiling all known microRNAs from a small volume of serum (<15 µL). NIHR funding has been secured for whole cohort analysis.

Results: Resulting data has shown very strong associations (up to $p < 10^{-12}$) for biological ageing, with 84 microRNAs meeting p -values < 0.05 (see heat map). Analysing the whole cohort will independently validate and extend the findings, in order to identify an ageing signature; the molecular clock.

Conclusions: This study suggests very strong changes in microRNAs in individuals between 50 and 62, suggesting microRNA signature is a molecular clock. These observations need to be confirmed and extended to validate serum microRNAs as biomarkers for ageing, for early detection of age-related disease and as tools to monitor ageing trajectory.

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THU0020

TOCILIZUMAB DECREASES THE PRO-INFLAMMATORY ROLE OF PLATELETS IN RHEUMATOID ARTHRITIS: IDENTIFICATION OF A NEW MECHANISM OF ACTION ASSOCIATED WITH POSITIVE RESPONSE?

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Background: Tocilizumab (TCZ), a humanised monoclonal antibody directed against IL-6 receptor, is an efficient treatment for rheumatoid arthritis (RA) but its mechanisms of action are not yet well understood.

Objectives: To identify new mechanisms of action of TCZ, by the study of gene expression fluctuations, between baseline (BL) and 3 months of treatment (T_{3m}), in RA patients.

Methods: TOSCA study has been realised to evaluate efficiency and tolerance of TCZ administered subcutaneously in active RA patients. Among the 125 patients of TOSCA study, 38 were ranked according to their treatment response after 3 months: 29 good responders (GR), 19 of which were also treated by methotrexate (MTX) and 10 non responders (NR), 7 of which were treated by MTX (GR: DAS 28-ESR_{3m} < 3.2 and Delta DAS28-ESR_(BL-3m) ≥ 3; NR: DAS 28-ESR > 3.2 and Delta DAS28-ESR_(BL-3m) < 3). A transcriptomic analysis was performed using a 44K whole human genomic DNA microarray (Agilent) on whole blood cells collected at BL and at T_{3m}. We identified genes with statistically significant expression fluctuations between BL and T_{3m} specifically in GR group (and not in NR group), treated by TCZ in monotherapy (excluding genes which fluctuated only with the association TCZ-MTX). Functional bio-informatics analysis was applied to this set of transcripts, by interrogation of Gene Ontology database, using Single Experiment Analysis tool and Natural Language Processing algorithms.

Results: Overall, 1089 transcripts significantly dysregulated were identified only in GR group at T_{3m} (t test, $p < 0.05$). This first set of transcripts was reduced to 783 by exclusion of transcripts that were fluctuated specifically when MTX was associated with TCZ in GR group. The functional analysis with these 783 genes dysregulated under TCZ in monotherapy enabled the identification of 8 transcripts (*CLU*, *F13A1*, *ITGA2B*, *ITGB3*, *SELP*, *SNCA*, *SPARC*, *TREML1*) whose relative abundances were significantly reduced at T_{3m}. These genes were enriched in "platelet alpha granule" GO functional category. Proteins encoded by these genes, either released in blood circulation or expressed at the cell membrane in case of platelet activation, have a pro-inflammatory activity through an interaction between platelets and immune cells.

Conclusions: This transcriptomic analysis suggests a new mechanism of action of TCZ in RA and the importance of platelets activation in RA pathophysiology. Indeed, genes linked with the pro-inflammatory role of platelets were down regulated. Further functional studies will be necessary to validate the direct effect of TCZ on platelets in RA.

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THU0021

DIFFERENTIAL EFFECTS OF TR14 VERSUS DICLOFENAC ON COX/LOX PATHWAYS REVEALED BY RNASEQ

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Background: Anti-inflammatory agents are used widely in treating numerous pain and inflammatory conditions. With a focus on the COX/LOX pathways in cutaneous wound repair in mice, the therapeutic activities of Tr14 (Traumeel), a multicomponent/multitarget natural product, and diclofenac (NSAID), a non-selective cyclooxygenase (COX) inhibitor were compared. The COX enzymes convert arachidonic acid into prostaglandins and thromboxanes, while the lipoxygenase (LOX) pathway generates more pro-inflammatory leukotrienes. Differential effects were identified via transcriptome analysis (RNAseq).

Objectives: To compare the transcriptomic changes after administration of Tr14 or diclofenac in a mouse cutaneous wound healing model, with particular emphasis on the COX/LOX pathway.

Methods: After abrasive wounding, the wounds were treated with topical Tr14 (34 mg/ml) in combination with subcutaneous Tr14 injections (9.5 mg/ml), or with subcutaneous Tr14 injections only, or topical diclofenac at clinically relevant doses (2 mg/ml). Skin samples were analysed for RNA transcript profiling by RNAseq at specific times (12 hour, 24 hour, 36 hour, 72 hour, 96 hour, 120 hour, 192 hour) after injury. Differentially expressed genes (DEGs) were computed at each time point between diclofenac vs control or Tr14 vs control, using EdgeR.

Results: At early time points (12–36 hour), both control and Tr14-treated wounds showed marked increase in the inducible COX2 enzyme mRNA, while diclofenac-treated wounds did not, likely due to blocking the PGE2 necessary for the feedback induction. Tr14, in contrast, had a striking inhibitory effect on mRNA levels