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OPEN Serum snoRNAs as biomarkers for joint ageing and post traumatic osteoarthritis

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The development of effective treatments for the age-related disease osteoarthritis and the ability to predict disease progression has been hampered by the lack of biomarkers able to demonstrate the course of the disease. Profiling the expression patterns of small nucleolar RNAs (snoRNAs) in joint ageing and OA may provide diagnostic biomarkers and therapeutic targets. This study determined expression patterns of snoRNAs in joint ageing and OA and examined them as potential biomarkers. Using SnoRNASeq and real-time quantitative PCR (gRT-PCR) we demonstrate snoRNA expression levels in murine ageing and OA joints and serum for the first time. SnoRNASeq identified differential expression (DE) of 6 snoRNAs in young versus old joints and 5 snoRNAs in old sham versus old experimental osteoarthritic joints. In serum we found differential presence of 27 snoRNAs in young versus old serum and 18 snoRNAs in old sham versus old experimental osteoarthritic serum. Confirmatory gRT-PCR analysis demonstrated good correlation with SnoRNASeg findings. Profiling the expression patterns of snoRNAs is the initial step in determining their functional significance in ageing and osteoarthritis, and provides potential diagnostic biomarkers and therapeutic targets. Our results establish snoRNAs as novel markers of musculoskeletal ageing and osteoarthritis.

Osteoarthritis (OA) is an age-related musculoskeletal disease and a common cause of chronic disability worldwide¹. In addition it is a significant contributor to both individual and socioeconomic burden and the number of disability adapted life years globally². If the deterioration in musculoskeletal health and development of OA can be identified and treated early serious life impairment may be abrogated. Ageing is the time-dependent reduction of functional capacity and stress resistance, associated with an increased risk of morbidity and mortality. The joint and its articular cartilage is particularly affected by ageing³. There is evidence that the rate of ageing, that is the 'biological age', differs significantly between individuals' actual age in years (i.e. the 'chronological age'). Defining markers of joint ageing may enable a prediction of the risk of onset of OA, enabling early intervention. OA is characterised by a non-symptomatic, pre-radiographical phase that if identified would allow earlier diagnosis. However radiographic changes are only evident later in disease progression. Magnetic resonance imaging techniques have been developed for early-stage evaluation of cartilage damage in OA but are expensive and contraindicated in some individuals.

The development of effective treatments for OA and the ability to predict disease progression has been hampered by the lack of substantive biomarkers, able to demonstrate pathological disturbances preceding identifiable tissue alterations. Others have attempted to identify products of tissue turnover in serum and synovial fluid (reviewed⁴). This has been challenging due to patient and disease heterogeneity and dilution effects either by tissue fluids or with similar products from other joints or diseases. In addition, the variability of antibody assays has been problematic.

SnoRNAs are a class of evolutionary conserved non-coding small guide RNAs of which the majority direct the chemical modification of other RNA substrates, including ribosomal RNAs and spliceosomal RNAs. In addition, some snoRNAs are involved in the regulation of alternative splicing and post-transcriptional modification of

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mRNA, whilst others exhibit miR-like activity⁵. Aberrant expression of snoRNAs has been associated with disease development⁵ such as lung tumorigenesis⁶.

Emerging evidence shows that there is an increased level of circulating RNAs in the serum of cancer patients⁷. Circulating microRNAs (miRs) have been extensively described as biomarkers for diseases like pancreatic/breast cancer^{8,9}, Alzheimer's disease¹⁰ and inflammatory diseases like asthma, inflammatory bowel disease and rheumatoid arthiritis¹¹, but with the recent discovery of stable¹² snoRNAs in serum, interest in their potential as circulating biomarkers of cancers (reviewed⁵) has been stimulated. We have previously identified dysregulation of a defined set of snoRNAs in cartilage¹³ and tendon¹⁴ ageing and OA¹⁵ and in man, snoRNA SNORD38 and SNORD48 were identified as potential non-age-dependant serum biomarkers for OA progression following cruciate ligament injury¹².

Expression profiling of snoRNAs in ageing and OA may help in determining their functional significance in the development and progression of disease and provide much needed diagnostic biomarkers for ageing and OA development. This study compared serum and joint snoRNA expression in ageing and OA from knee joint tissues from young and old adult mice and old mice using a traumatic *in vivo* model of OA. Because OA involves the whole joint as an organ; we undertook our analysis on whole mouse joints, which included cartilage, meniscus, subchondral bone, and joint capsule with synovium.

Materials and Methods

All reagents were from Thermo-Fisher-Scientific, unless stated.

Animals. C57BL6/J male mice were used for the study. For SnoRNASeq old mice were 18 months old (n = 6), young 8 months old $(n = 6)^{16}$ and mice used for destabilisation of the medial meniscus (DMM) 24 months old (sham n = 3; DMM n = 6). Mice were group housed in individually ventilated cages at a 12 hour light/dark cycle, with *ad libitum* access to food and water. Experimental animal protocols were performed in accordance with the guidelines of the Animals (Scientific Procedures) Act 1986 following ethical review. Animal usage and protocols for this study was approved by the University of Liverpool Animal Welfare Committee.

Surgical induction of OA by DMM in mice. DMM surgery was perform as previously reported¹⁷. Briefly, under anaesthesia a 3 mm skin incision was made over the medial aspect of the patellar ligament through the joint capsule into the femorotibial joint of the left knee. The medial meniscotibial ligament was transected to destabilise the cranial pole of the medial meniscus from the anterior tibial plateau. In sham operated mice the medial meniscotibial ligament was visualised but not transected. Mice were sacrificed 8 weeks post-surgery.

Joint and serum collection for SnoRNASeq. Following euthanasia, knee joints were collected from young, old, DMM (n = 6 each group) and sham n = 3 for SnoRNASeq. Joints were harvested free of soft tissues at 7 mm from the joint into RNALater. Serum was collected using cardiac puncture. One old serum sample was not processed further due to extensive haemolysis.

OARSI scoring of histological sections of mouse knee joints. For histology, as the total knee joint was used in the study for RNA extraction, joints were collected (into 4% paraformaldehyde) from additional equivalent aged and treated young (n = 8), old (n = 4); sham (n = 5); and DMM (n = 6) mice in order to evaluate the extent of OA. The procedure, surgeon and duration of the studies were identical. Knees were decalcified in 0.5 M ethylenediaminetetraacetic acid (pH 7.4) for 4 weeks at 4 °C and coronally embedded in paraffin. Sectioning, Safranin-O Fast-Green staining and histological scoring (defined as the severity and extent of OA) was undertaken on a scale from 1 to 6 by two blinded independent observers using the OARSI histopathology initiative¹⁸. All four quadrants of the section (medial tibial plateau, lateral tibial plateau, medial femoral condyle, lateral femoral condyle) were scored individually and added for each histological section. For statistical analyses mean summed score values of joints of 3–5 section per knee joint at 4 depths throughout the joint was determined (thus a maximum score of 24 was possible). Inter-observer variability was calculated using Cohen's Kappa statistics using an online software tool: (http://www.statstodo.com/CohenKappa).

RNA isolation, RNA-Seq analysis, cDNA library preparation and sequencing. Total RNA was isolated from equal weights of joints and 500µl serum using miRNeasy or RNeasy Serum kits respectively with DNase treatment (all Qiagen, Crawley, UK) to remove residual gDNA. Total RNA integrity (RIN) was confirmed using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA). Ribosomal RNA was depleted using the Ribo-Zero[™] rRNA Removal Kit (Epicentre, Madison, USA). From 41 samples 100 ng of rRNA-depleted RNA was submitted for library preparation using NEB small RNA library kit (New England Biolabs (NEB), Ipswich, USA). To reduce workflow bias we used tobacco acid pyrophosphatase (Epicentre, Madison, USA) to remove potential 5′ caps found on some snoRNAs. Samples were amplified for 15 cycles, mixed into 3 pools, and size selected. The size-selected material was purified with Ampure beads (Agencourt, Beckman-Coulter, High-Wycombe, UK). SnoRNA sequencing was undertaken on the Illumina HiSeq 2000 platform (Illumina, San Diego, USA) using 100 base paired-end reads.

SnoRNASeq data analysis. Sequence data measured from 5 lanes of an Illumina HiSeq2000 were processed through a number of steps to obtain snoRNA expression values. The processes include basecalling and de-multiplexing of indexed reads using CASAVA version 1.8.2¹⁹; adapter and quality trimming using Cutadapt version 1.2.1²⁰ and Sickle version 1.200 to obtain fastq files of trimmed reads; aligning reads to Ensembl GRCm38.77 mouse genome reference sequences which contains 1,555 annotated snoRNA features using Bowtie2²¹ version 2.0.10 with option "-very-sensitive-local"; counting aligned reads against snoRNA features using THSeq-count. The count values were used as snoRNA expression measurements for the DE analysis.

Name	Sequence (5'-3')	Tm (°C)
RTQ primer poly(A) cDNA synthesis	CGAATTCTAGAGCTCGAGGCAGGC GACATGGCTGGCTAGTTAAGCTTG GTACCGAGCTCGGATCCACTAGTCC TTTTTTTTTT	75
Snora28	CATGAGACAAGCCGTTATATAGGC	50
Snora30	TGTACCAGTGGCAGCTGTTACTC	50
Snora31	CTTTGTGGCAGTTCAGATTGAATTAG	50
Snora64	GTGGCCTCTCTTGCCTAGAG	65
Snora73	ACAGTGACTGAGGAGGCAAAC	50
Snord46	AATGCAAGGACTTGTCATAGTTACAC	50
Snord85	TTAGACCAGAGGTCGATGATGAG	50
Snord88	ACCTTTGACACCTGGAGATCTGA	50
Snord116	TGTACCGCCACTCTCATCGG	65
U2	TGGTATTGCAGTACCTCCAGGAACG	55
U3	AGTGAGAGGGAGAGAACGCGGTC	55
U6	GATGACACGCAAATTCGTGAAGCGTTC	55
RTQ-UNIr-50	AATTCTAGAGCTCGAGGCAGG	50
RTQ-UNIr-55	CGAATTCTAGAGCTCGAGGCAGG	55
RTQ-UNIr-65	CTAGAGCTCGAGGCAGGCGACATGGCTGGC	65

Table 1. Oligonucleotides sequences used in qRT-PCR.

DE analysis was performed in R environment using package edgeR²². The processes and technical details of the analysis include: assessing data variation and detecting outlier samples through comparing variations of within and between sample groups using principle component analysis (PCA; 3-D PCA plots were generated using R function in package plot3D) and correlation analysis; handling library size variation respectively for joint samples and serum samples through data normalisation; formulating data variation using negative binomial distributions; modelling data using a generalised linear model; computing log₂ Fold Change (logFC) values for required contrasts based on model fitting results through contrast fitting approach, assigning P-values to logFC values by LR²³ testing; dealing with the effects of multiple tests using FDR approach to obtain FDR adjusted P-values; and defining significantly DE snoRNAs as those with FDR-adjusted p-value < 5%. Sequence data have been submitted to National Centre for Biotechnology Information Gene Expression Omnibus (NCBI GEO); E-MTAB-4878.

RNA isolation, poly(A) cDNA synthesis and snoRNA qRT-PCR. qRT-PCR of snoRNAs was performed²⁴. Total RNA was isolated using the mirVana kit. Isolated RNA samples were polyadenylated at 37 °C for 60 minutes in a 50 µL reaction volume containing 1 µg RNA and 1.5 U poly(A) polymerase (NEB, Ipswich, USA). 500 µL lysis binding buffer was added. Then, an equal volume of acid-phenol:chloroform was added, vortexed and samples were centrifuged for 10 minutes and the aqueous phase removed. The poly(A)-tailed total RNA was extracted using the filter cartridge provided by the mirVana kit. To generate poly(A) cDNA, 500 ng poly(A)-tailed RNA and 250 ng RTQ primer (Eurogentec, Seraing, Belgium) (Table 1) were mixed in a 26µL reaction volume, incubated at 65 °C for 10 minutes and annealed at 4 °C for 20 minutes. Reverse transcription was performed with 200 U M-MLV reverse transcriptase, 20 U RNAsin (both Promega, Southampton, UK), 2 µL dNTP mix (10 mM each; Eurogentec, Seraing, Belgium) and 8µL 5x M-MLV buffer (Promega, Sothampton, USA) in a total reaction volume of $40\,\mu$ L at 50 °C for 60 minutes. The reverse transcriptase was inactivated at 70 °C for 15 minutes. Finally, 1.5 U of RNAse H (NEB, Ipswich, USA) was added to remove small RNAs. A snoRNA-specific forward primer and a universal reverse primer (RTQ-UNIr, matched to the Tm of each individual snoRNA) were used for the amplification of each snoRNA target (all Eurogentec, Seraing, Belgium) (Table 1). For each cDNA sample a mix was prepared with Mesagreen qPCR Mastermix Plus for SYBR Green (Eurogentec, Seraing, Belgium) and 300 nM forward and reverse oligonucleotides. An ABI-7300 Detection System was used for amplification using the following protocol: denaturation at 95 °C for 5 minutes, followed by 50 cycles of DNA amplification (15 seconds 95 °C and 45 seconds annealing at 62-68 °C). The annealing temperature was optimized for each snoRNAs target. Serially diluted standard curves were utilized to quantify snoRNA expression and data was normalized to a validated housekeeping snoRNA (joint: U2, young-old serum: SNORD85, old sham-old DMM serum and equine serum: U6).

Validation of SNORD116 as a marker of OA in equine serum. We determined the reproducibility of the expression of SNORD116 in OA serum of another species. There are well-published studies on the application of metacarpophalangeal (MCP) joint OA changes in the horse, a joint with similarities to the human knee joint²⁵. We studied equine serum from normal and MCP OA horses collected from eight normal (mean age \pm standard deviation 5.3 ± 2.1 years) and four OA (7.5 ± 1.0 years) castrated male thoroughbred horses at post-mortem. Samples were collected under the regulations of the Hong Kong Jockey Club with owner consent and stored at -80 °C. OA diagnosis was based on histological (modified Mankin)²⁶ and synovitis scoring²⁷ of MCP joint tissues.



Figure 1. Histological changes of in the mouse knee showing the medial femoral condyle (above) and medial tibial plateau (below). (A) Safranin O with Fast-Green counterstain. Scale bar, $100 \mu m$. Red indicates proteoglycan. (B/C) Assessment of osteoarthritis development was evaluated by OARSI scores; young (n = 8) vs. old (n = 4) (B); sham (n = 5) vs. DMM (n = 6) (C). Data represents the mean + 95% confidence interval (CI) for each scorer. For statistical evaluation an independent samples t-test was performed using GraphPad Prism 5 (San Diego); p-values are indicated.

Statistical analysis. For statistical evaluation of histological scoring non-parametric Mann-Whitney-U test was used. Inter-observer agreement of histological scoring systems was calculated using Cohen's kappa coefficient (www.statstodo.com/CohenKappa_Pgm.phpl). qRT-PCR data was log-transformed prior to statistical evaluation with an independent samples t-test. Statistical evaluation was performed between young and old or old sham versus old DMM using GraphPad Prism 5 (San Diego); p-values are indicated.

Results

OARSI scoring of joints. OARSI scoring of joints (mean \pm 95%CI) for young and old were 0.5 \pm 0.3 and 2.8 \pm 2.7 (p = 0.01), and old sham and old DMM were 1.25 \pm 1.1 and 6.5 \pm 0.7 (p < 0.001), respectively. Mice exhibited typical histological features of OA in the DMM knees. Cohen's Kappa statistic was 0.4 indicating a fair agreement. Representative histological images and OARSI scoring are in Fig. 1.

Preliminary analysis SnoRNASeq. To identify DE of snoRNAs in mouse joints and serum in response to age and OA 41 cDNA libraries representing old and young joints (old = HJO; young = HJY) and serum (old = HSO; young = HSY), and old sham and old DMM joints and serum (sham joint = DJC, DMM joint = DJM, sham serum = DSC and DMM serum = DSM) were constructed and subjected to Illumina deep sequencing. Summaries of raw, trimmed reads and sequencing alignment to mouse snoRNAs are in Supplementary files 1 and 2 respectively. Reads mapping percentages for joint libraries were between 12~29%, and for serum libraries 0.03~0.9%. Between 42~53% of the 1555 mouse snoRNA reference sequences were aligned for joints and 16~26% for serum.

Identification of DE snoRNAs using SnoRNASeq. The 3-D PCA plot (Fig. 2A) shows that the joint samples and serum samples are clearly separated by the 1st component, which explains 93.02% of the data variation. For serum samples, the group sham and the group DMM scatter separately on the 2nd component, furthermore, based on the 3rd component a clear separation between serum samples of young-healthy and samples of old-healthy exists. For joint samples, there is also a separation between DMM and sham samples, though the separation is not as clear as shown for serum samples. In addition, 5 DMM joint samples scatter far away from other joint samples on the 3rd component. Therefore, it can be expected that disease response small RNA can be detected from this data set. The heat map of hierarchical clusters of correlations among samples (Fig. 2B) depicts that the joint and serum groups of samples are very different in snoRNA expression. In addition, joint samples correlated to each other much more closely than serum samples confirming the phenomena revealed by the PCA analysis shown in Fig. 2A. This indicates that major disease responses in the data were contributed from serum samples. A read length distribution graph was generated to highlight the constitutional difference of non-coding RNAs in joint and serum samples at 100 bp or below (Fig. 2C). For this one serum and one joint sample with approximately the same library size were chosen. The plots of the read length distribution for joint and serum



Figure 2. Variation data between the expressions for 41 samples. (A) A 3-D PCA plot of the first three components from principal component analysis of logarithm-transformed small RNA abundance data. Variance (%) associated with each principle component is depicted on the respective axis. Abbreviations; Joint: young healthy (HJY; black), old healthy (HJO; red), old sham (DJC; magenta), old DMM (DJM; light blue). Serum: young healthy (HSY; green), old healthy (HSO; dark blue), old sham (DSC; grey), old DMM (DSM; yellow). (B) The heat map of hierarchical clusters of correlations among samples. Pearson's correlation coefficients were computed using logarithm transformed small RNA expression data from all known snoRNAs that were detected. (C) The plots of read length distribution for two representative joint and serum samples.

samples reveal that a peak value of frequency for read length in joint samples is 22 bp (vertical, dotted black line). Therefore the corresponding reads are generally well annotated miRNAs. Another peak is noted at around 100 bp, which is consistent with non-coding RNAs whose length is a hundred bp and over. In contrast, a peak is presented at 30 bp long for serum samples (vertical, dotted red line). More than 50% of the reads are 30 bp long in the serum library (data not shown). In contrast, the joint library has just 1.14% of reads that are 30 bp long (data not shown). Such reads may come from piwi-interacting RNAs (piRNAs). There were 498–646 snoRNAs expressed in serum samples and 1068–1286 in joint samples. The DE snoRNAs between contrasts are in Table 2. These included 6 snoRNAs in young versus old joints, 5 snoRNAs in old sham versus old DMM joints. In serum we identified DE of 27 snoRNAs in young versus old serum and 18 snoRNAs in old sham versus old DMM serum.

Validation of SnoRNASeq data using qRT-PCR. Very little is known about the roles of snoRNAs as biomarker for disease or about the functional roles of snoRNAs in mammalian cell biology. We thus could not define functional criteria to select snoRNAs for validation based on prior knowledge. To stay unbiased we thus selected snoRNAs from the RNAseq data that displayed a moderate fold significant expression difference, higher fold significant expression difference, non-significant expression difference and selected box H/ACA (SNORAs) as well as box C/D (SNORDs) snoRNAs for validation. Levels of candidate snoRNAs for further qRT-qPCR analysis were determined using the original RNA from all donors used to perform the SnoRNASeq experiment. There was good concordance between SnoRNASeq and qRT-PCR platforms (Table 2 and Fig. 3). SNORD88

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Image: style		ENSMUSG0000095118	SNORD14D	1.23	0.00	Heat shock protein 8
ENSAUSC0000006987 SNORA17 1.90 0.01 Predicted gene ENSAUSC000006493 SNORA18 1.99 0.00 ARB. allystation repair homolog 1 ENSAUSC000006493 SNORA13 2.12 0.00 Regulator of chromosome condeniation 1 ENSAUSC000006493 SNORA13 2.14 0.01 Regulator of chromosome condeniation 1 ENSAUSC0000064918 SNORA13 2.14 0.02 Ribonnal protein 1A ENSAUSC00000064918 SNORA23 2.26 0.02 Ribonnal protein 1A ENSAUSC00000064918 SNORA23 2.39 0.03 Enkaryotic translation initiation factor 5 ENSAUSC0000006495 SNORA53 2.49 0.00 Stotaryotic translation initiation factor 5 ENSAUSC0000006495 SNORA53 2.49 0.00 Regulator of chromosome condeniation 1 and an Inc ENSAUSC0000006495 SNORA53 3.00 0.00 Neclosin ENSAUSC0000006497 SNORD17 2.56 0.00 Neclosin ENSAUSC0000006497 SNORA53 3.00 0.00 Nuclosin ENSAUSC000000		ENSMUSG0000065219	SNORD32A	1.55	0.04	Ribosomal protein L13A
FINSMUSC0000006428 mR3068/NNRA58 1.96 0.00 AlkB, alkylation repark homolog i FINSMUSC000000528 SNORA14 2.02 0.01 Regulator of chromosome condensation 1 ENSMUSC0000006402 SNORA14 2.12 0.00 Beakaryotic translation clongation factor 1 beta 2 ENSMUSC0000006402 SNORA13 2.14 0.03 Regulator of chromosome condensation 1 ENSMUSC00000064018 SNORA73 2.26 0.02 Ribosomal protein L4 ENSMUSC00000064018 SNORA5 2.39 0.03 Eukaryotic translation initiation factor 5 ENSMUSC00000064918 SNORA5 2.49 0.00 Transforming growth factor beta regulated gene 4 ENSMUSC00000004791 SNORA5 2.49 0.00 Transforming growth factor beta regulated gene 4 ENSMUSC00000004791 SNORD17 2.76 0.00 Adaptor -related protein complet 1, gamma 1 submit ENSMUSC00000004791 SNORD20 3.09 0.00 Nucleoin ENSMUSC00000064575 SNORA17 1.14 0.00 Hypothetici protein PNA5-123 ENSMUSC00000064576 SNORA23 <		ENSMUSG0000088678	SNORA17	1.90	0.03	Predicted gene
FINSMUSC0000006452 SNORA18 1.9 0.00 Predictor gene FNSMUSC0000006487 SNORA73A 2.02 0.00 Regulatory associated protein of MTOR, complex 1 ENSMUSC0000006402 SNORA13 2.14 0.03 Regulatory associated protein of MTOR, complex 1 ENSMUSC0000006405 SNORA28 2.39 0.03 Eukaryotic translation initiation factor 5 ENSMUSC0000007764 SNORA28 2.39 0.03 Eukaryotic translation initiation factor 5 ENSMUSC0000007764 SNORA53 2.49 0.00 Transforming growth factor beta regulated genet ENSMUSC00000064569 SNORA53 2.49 0.00 Anterior family 2.5 ENSMUSC00000064569 SNORD17 2.76 0.00 Anterior family 2.5 ENSMUSC00000065774 SNORD23 4.42 0.00 Nucleolin ENSMUSC00000066578 SNORA23 4.32 0.00 Nucleolin ENSMUSC00000066577 SNORA55 -1.00 Ginma tumor suppressor candidate region gene 2 ENSMUSC00000066578 SNORA23 1.340 0.00 Hypothetical protein Atsocial protein		ENSMUSG0000064938	miR3068/SNORA58	1.96	0.00	AlkB, alkylation repair homolog 1
Enskut/Sci0000064387 SNORA73A 2.02 0.01 Regulator of chromosome condensation 1 INSMUSG000006402 SNORA31 2.12 0.00 Ekaryotic translation elongation factor 1 beta 2 ENSMUSG000006518 SNORA31 2.14 0.03 Regulatory associated protein of MTOR, complex 1 ENSMUSG000006518 SNORA73 2.26 0.03 Eukaryotic translation initiation factor 5 ENSMUSG0000064493 SNORA73 2.43 0.00 Solute carrier family 25 ENSMUSG0000064493 SNORA73 2.43 0.00 Transforming growth factor beta regulated gene 4 ENSMUSG0000064493 SNORD71 2.56 0.00 Adaptor related protein complex 1, gamma 1 subunit ENSMUSG0000064956 SNORD71 2.76 0.00 Sorting nexin 5 ENSMUSG00000064971 SNORD73 3.00 O Nucleoin ENSMUSG00000064975 SNORD73 3.00 O Nucleoin ENSMUSG0000006476 SNORD73 3.00 Bynobioticit protein AS+123 ENSMUSG000006477 SNORD73 1.00 Bynobioticit protein PMAS+123		ENSMUSG0000065282	SNORA18	1.99	0.00	Predicted gene
FINMUSC0000006402 SNORA41 2.12 0.00 EbsArryotic translation indengation factor 1 beta 2 FONMUSC00000064018 SNORA13 2.14 0.03 Regulatory associated protein of MTOR, complex 1 FONMUSC000000641918 SNORA73 2.26 0.02 ERSAUSC000011-like beta FONMUSC00000064195 SNORA53 2.43 0.00 Solute carrier family 25 FONMUSC00000064195 SNORA51 2.49 0.00 Transforming growth factor beta regulated gene 4 FONMUSC00000064195 SNORA51 2.49 0.00 Adaptor-related protein factor beta regulated gene 4 FONMUSC00000064195 SNORD14E 2.63 0.00 Heat shock protein 8 FONMUSC00000064791 SNORD17 2.76 0.00 Nucleoin FONMUSC00000065778 SNORD73B 3.10 0.02 Poly(A) binding protein, cytoplasmic 4 FONMUSC00000065776 SNORA53 3.10 0.02 Poly(A) binding protein, cytoplasmic 4 FONMUSC00000065776 SNORA21 13.40 0.00 Hypothetical protein PNA5-123 FONMUSC0000006575 SNORA35 -0.88		ENSMUSG0000064387	SNORA73A	2.02	0.01	Regulator of chromosome condensation 1
First ENSMUS ENSMUS Ensmus Regulatory association for MTOR, complex 1 Young vs. Old serum ENSMUS SNORA13 2.14 0.03 Regulatory association initiation factor 5 ENSMUS ENSMUS SNORA23 2.26 0.02 ENO-1-like beta ENSMUS ENSMUS SNORA53 2.24 0.00 Solut carrier family 25 ENSMUS SNORA55 2.49 0.00 Solut carrier family 25 ENSMUS SNORA57 2.49 0.00 Adaptor-related protein complex 1, gamma 1 subunit ENSMUS SNORA71 2.56 0.00 Adaptor -related protein complex 1, gamma 1 subunit ENSMUS SNORA73 2.79 0.00 Regulator of chromosome condensation 1 and an Inc ENSMUS ENSMUS SNORA53 3.10 0.02 Poly(A) binding protein, cryoplasmic 4 ENSMUS ENSMUS SNORA53 3.42 0.00 Hypothetical protein PNA5-123 ENSMUS ENSMUS SNORA53 -1.24 0.00 Hypothetical protein /14 ENSMUSC00000069176 SNORA54 <td></td> <td>ENSMUSG0000064602</td> <td>SNORA41</td> <td>2.12</td> <td>0.00</td> <td>Eukaryotic translation elongation factor 1 beta 2</td>		ENSMUSG0000064602	SNORA41	2.12	0.00	Eukaryotic translation elongation factor 1 beta 2
EINSMUSC00000064918 SNORA73 2.18 0.02 RBbonal protein 1.4 Young vs. Old serum ENSMUSC0000064918 SNORA73 2.26 0.03 Eukaryotic translation initiation factor 5 ENSMUSC0000006493 SNORA73 2.47 0.00 Transforming growth factor beta regulated gene 4 ENSMUSC0000006495 SNORA71 2.56 0.00 Adport-related protein complex 1, gamma 1 submit ENSMUSC00000064791 SNORD14 2.63 0.00 Heat shock protein 8 ENSMUSC00000064791 SNORD20 3.09 0.00 Nucleolin ENSMUSC00000064796 SNORD20 3.09 0.00 Nucleolin ENSMUSC00000064776 SNORD20 3.09 0.00 Nucleolin ENSMUSC00000064776 SNORD23 4.22 0.00 Glomat umor suppressor candidate region gene 2 ENSMUSC00000064778 SNORD23 4.42 0.00 Hypothetical protein, stpaat ENSMUSC00000064791 SNORA17 1.14 0.00 Hypothetical protein, stpaat ENSMUSC00000064515 SNORA44 5.65 0.00 Hypothetical pr		ENSMUSG0000088670	SNORA31	2.14	0.03	Regulatory associated protein of MTOR, complex 1
Young vs. Old serum ENSMUSC00000065158 SNORA73 2.26 0.02 ERC1-like beta ENSMUSC00000064493 SNORA53 2.39 0.03 Eukaryotic translation initiation factor 5 ENSMUSC00000064493 SNORA53 2.43 0.00 Solute carrier family 25 ENSMUSC00000064509 SNORD71 2.56 0.00 Adaptor-related protein complex 1, gamma 1 subuit ENSMUSC000000645701 SNORD71 2.76 0.00 Regulator of chromosome condensation 1 and an Inc ENSMUSC00000065773 SNORD20 3.09 0.00 Nocloclin ENSMUSC00000065778 SNORD23 4.42 0.00 Glioma tumor suppressor candidate region gene 2 ENSMUSC00000065776 SNORA14 5.65 0.00 Hypothetical protein, cytoplasmic 4 ENSMUSC0000006575 SNORA2 13.40 0.00 Hypothetical protein NSC123 ENSMUSC0000006575 SNORA2 13.40 0.00 Hypothetical protein, istaa ENSMUSC0000006455 SNORA2 13.40 0.00 Hypothetical protein, istaa ENSMUSC00000006455 SNORA29 -1.12		ENSMUSG0000064918	SNORD18	2.18	0.02	Ribosomal protein L4
ENSMUSC60000006449 SNORA28 2.39 0.03 Eukaryotic translation initiation factor 5 ENSMUSC60000064495 SNORA53 2.43 0.00 Transforming growth factor beta regulated gene 4 ENSMUSC60000064495 SNORD1 2.56 0.00 Adaptor-related protein complex 1, gamma 1 subunit ENSMUSC60000064791 SNORD14E 2.63 0.00 Regulator of chromosome condensation 1 and an Inc ENSMUSC60000064791 SNORD20 3.09 0.00 Regulator of chromosome condensation 1 and an Inc ENSMUSC6000006466 SNORD20 3.09 0.00 Regulator of chromosome condensation 1 and an Inc ENSMUSC60000006478 SNORD23 4.42 0.00 Glioma tumor suppressor candidate region gene 2 ENSMUSC60000006478 SNORA17 12.14 0.00 Hypothetical protein NC616037, homology human collagen alpha 2 ENSMUSC60000006453 SNORA2 13.40 0.00 Hypothetical protein, 152aa ENSMUSC60000006453 SNORA3 -1.12 0.04 Lnc-Na imprinted and accumulated in nucleus Sham vs. DMM joit ENSMUSC60000007571 SNORA56 -0.48 0.05	Young vs. Old serum	ENSMUSG0000065158	SNORA73	2.26	0.02	ERO1-like beta
ENSMUSC600000064495 SNORA53 2.43 0.00 Storming growth factor beta regulated gene 4 ENSMUSC60000064495 SNORA5 2.49 0.00 Transforming growth factor beta regulated gene 4 ENSMUSC600000064591 SNORD11 2.56 0.00 Advort-related protein complex 1, gamma 1 subunit ENSMUSC60000005771 SNORD178 2.76 0.00 Regulator of chromosome condensation 1 and an Inc ENSMUSC60000005778 SNORA55 3.10 0.02 Poly(A) binding protein, cryoplasmic 4 ENSMUSC60000005776 SNORA44 5.55 0.00 Glioma tumor suppressor candidate region gene 2 ENSMUSC0000000576 SNORA44 5.55 0.00 Hypothetical protein, PNA5-123 ENSMUSC0000000576 SNORA44 5.55 0.00 Hypothetical protein, PNA5-123 ENSMUSC00000006582 SNORA17 12.14 0.00 Hypothetical protein, PNA5-123 ENSMUSC000000064585 SNORA43 -1.12 0.04 Lnc-snall nucleadra RNA host gene 7 ENSMUSC00000064585 SNORA43 -1.12 0.04 Lnc-snall nuclear RNA host gene 7 ENSMUSC00000007719		ENSMUSG0000064493	SNORA28	2.39	0.03	Eukaryotic translation initiation factor 5
ENSMUSC00000064495SNORA52.490.00Transforming growth factor beta regulated gene 4ENSMUSC0000004569SNORD712.560.00Adaptor-related protein complex 1, gamma 1 subunitENSMUSC0000007714SNORD172.760.00Regulator fortomosome condensation 1 and an IncENSMUSC0000005353SNORD5382.790.00Regulator fortomosome condensation 1 and an IncENSMUSC0000005778SNORD553.100.02Poly(A) binding protein, cytoplasmic 4ENSMUSC0000005778SNORD234.420.00Glioma tumor suppressor candidate region gene 2ENSMUSC000000576SNORA445.550.00Hypothetical protein, MCG16937, homology humanENSMUSC0000005578SNORA55-0.800.40Hypothetical protein, cytoplasmic 4ENSMUSC0000005582SNORA55-0.800.40Hypothetical protein, cytoplasmic 4ENSMUSC00000065852SNORA53-0.990.40Lnc- NA imprinted and accumulated in nucleusSham vs. DMM jointENSMUSC0000005455SNORA43-1.120.40T-complex protein, cytoplasmic 4ENSMUSC0000006488SNORA54-0.120.04T-complex protein 1ENSMUSC00000064919SNORA641.300.31Malten etapit homolog 1ENSMUSC00000064919SNORA643.300.31Predicted geneENSMUSC00000064918SNORA643.050.41Releven in binoucleoprotein NENSMUSC00000064918SNORA641.550.41Ribosomal protein 1.5ENSMUSC00000064918SNORA64 <td< td=""><td></td><td>ENSMUSG0000077167</td><td>SNORA53</td><td>2.43</td><td>0.00</td><td>Solute carrier family 25</td></td<>		ENSMUSG0000077167	SNORA53	2.43	0.00	Solute carrier family 25
ENSMUSC00000064599SNORD712.560.00Adaptor-related protein complex 1, gamma 1 subunitENSMUSG00000064791SNORD14E2.630.00Heat shock protein 8ENSMUSG00000064791SNORD73B2.760.00Regulator of chromosome condensation 1 and an IncENSMUSG00000065335SNORD203.090.00NucleolinENSMUSG00000065778SNORD234.420.00Gloma tumor suppressor candidate region gene 2ENSMUSG00000069176SNORA45.650.00Hypothetical protein PAS-123ENSMUSG0000005852SNORA1712.140.00Hypothetical protein PAS-123ENSMUSG00000065852SNORA213.400.00Hypothetical protein IS2aENSMUSG00000064855SNORA55-0.880.44Poly(A) binding protein, cytoplasmic 4ENSMUSG00000064855SNORA43-1.120.40Enc-Nitiprited and accumulated in nucleusENSMUSG00000064856SNORA43-1.120.44T-complex protein 1ENSMUSG00000064850SNORA43-1.120.44T-complex protein 1ENSMUSG00000064105SNORA55-0.120.44T-complex protein 1ENSMUSG00000064105SNORA56-1.620.44Small nuclear ribonucleoprotein NENSMUSG00000064105SNORA64-1.620.44Small nuclear ribonucleoprotein NENSMUSG0000006410SNORA64-1.620.41ENSMUSG000006416ENSMUSG0000006410SNORA64-1.620.41ENSMUSG000006416ENSMUSG0000006411SNORA64-1.62 <t< td=""><td rowspan="11"></td><td>ENSMUSG0000064495</td><td>SNORA5</td><td>2.49</td><td>0.00</td><td>Transforming growth factor beta regulated gene 4</td></t<>		ENSMUSG0000064495	SNORA5	2.49	0.00	Transforming growth factor beta regulated gene 4
ENSMUSC0000064791SNORD14E2.630.00Heat shok protein 8ENSMUSQ0000077714SNORD172.760.00Sorting nexin 5ENSMUSQ0000065353SNORD203.090.00NucleolinENSMUSQ000006578SNORD204.090.00RoburdENSMUSQ000006778SNORA553.100.02Poly(A) binding protein, cytoplasmic 4ENSMUSQ000008078SNORA1712.140.00Hypothetical protein PNAS-123ENSMUSQ0000080800SNORA212.140.00Hypothetical protein protein cytoplasmic 4ENSMUSQ000008080SNORA212.140.00Hypothetical protein cytoplasmic 4ENSMUSQ000008801SNORA2-0.880.04Hypothetical protein cytoplasmic 4ENSMUSQ000008802SNORA2-0.880.04Hopothylanding protein, cytoplasmic 4ENSMUSQ000008455SNORA2-1.220.04Lnc-Na imprinted and accumulated in nucleusENSMUSQ000006455SNORA2-1.220.04Creanel and accumulated in nucleusENSMUSQ000006455SNORA4-1.120.04Creanel and accumulated in nucleusENSMUSQ000006455SNORA5-0.280.04Horeintra complex protein 1ENSMUSQ000006455SNORA5-0.120.04Creanel and accumulated in nucleusENSMUSQ000006451SNORA64-1.620.01Horeintra complex protein 1ENSMUSQ000006451SNORA5-0.280.01Horeintra complex protein 1ENSMUSQ000006451SNORA51.350.01Horeintra complex prote		ENSMUSG0000064569	SNORD71	2.56	0.00	Adaptor-related protein complex 1, gamma 1 subunit
ENSMUSG000007714SNORD172.760.00Sorting nexin 5ENSMUSG0000005353SNORD73B2.790.00Regulator of chromosome condensation 1 and an IcuENSMUSG0000005466SNORD57B3.100.02Poly(A) binding protein, cytoplasmic 4ENSMUSG0000005778SNORA553.100.02Poly(A) binding protein, cytoplasmic 4ENSMUSG0000005166SNORA445.650.00Hypothetical protein PNAS-123ENSMUSG0000005167SNORA55-0.880.00Hypothetical protein PNAS-123ENSMUSG0000005852SNORA55-0.880.04Poly(A) binding protein, cytoplasmic 4ENSMUSG0000064585SNORA55-0.880.04Poly(A) binding protein, etylasmic 4ENSMUSG0000064585SNORA55-0.880.04Incr-snal nucleolar RNA host gene 7ENSMUSG0000064585SNORA55-1.220.04Incr-snal nucleolar RNA host gene 7ENSMUSG0000064585SNORA16-1.620.04Incr-snal nucleolar RNA host gene 7ENSMUSG0000064595SNORA160.150.01Intra-snal nucleolar RNA host gene 7ENSMUSG0000064598SNORA662.780.01Ribosomal protein 1.5ENSMUSG0000064591SNORA662.780.01Ribosomal protein 1.5ENSMUSG0000064591SNORA662.780.01Ribosomal protein 1.5ENSMUSG0000064591SNORA641.850.44Small nuclear ribonucleoprotein NENSMUSG0000064591SNORA641.850.40Small nuclear ribonucleoprotein NENSMUSG0000064593 </td <td>ENSMUSG0000064791</td> <td>SNORD14E</td> <td>2.63</td> <td>0.00</td> <td>Heat shock protein 8</td>		ENSMUSG0000064791	SNORD14E	2.63	0.00	Heat shock protein 8
ENSMUSG0000065353SNORD73B2.790.00Regulator of chromosome condensation 1 and an lncENSMUSG0000066666SNORD203.090.00NucleolinENSMUSG0000066778SNORA553.100.02Poly(A) binding protein, cytoplasmic 4ENSMUSG000000778SNORA445.650.00Hypothetical protein PAAS-123ENSMUSG00000080478SNORA1712.140.00Hypothetical protein MGC1007, homology human collagen alpha 2ENSMUSG0000068552SNORA213.400.00Hypothetical protein, 152aaENSMUSG00000068553SNORA55-0.880.04Poly(A) binding protein, cytoplasmic 4ENSMUSG0000006455SNORA55-0.880.04Poly(A) binding protein, cytoplasmic 4ENSMUSG0000006455SNORA55-0.880.04Enc-NA imprinted and accumulated in nucleusSham vs.DMM jointENSMUSG0000006455SNORA39-1.120.04Lnc-small nucleuralRNA host gene 7ENSMUSG00000064515SNORA54-1.620.04Small nuclear ribonucleoprotein NENSMUSG00000065171SNORA54-1.620.04Small nuclear ribonucleoprotein NENSMUSG00000064017SNORA563.310.03AlkB, alkylation repair homolog 1ENSMUSG0000064918SNORA161.850.01Predicted geneENSMUSG00000064918SNORA641.850.04Small nuclear ribonucleoprotein NENSMUSG0000064917U901.900.01ImportinENSMUSG0000064918SNORD48-1.000.04Rikbosomal protein 1.13 <td>ENSMUSG0000077714</td> <td>SNORD17</td> <td>2.76</td> <td>0.00</td> <td>Sorting nexin 5</td>		ENSMUSG0000077714	SNORD17	2.76	0.00	Sorting nexin 5
ENSMUSG0000064696SNORD203.090.00NucleolinENSMUSG0000064696SNORD233.100.02Poly(A) binding protein, cytoplasmic 4ENSMUSG00000080478SNORD234.420.00Glioma tumor suppressor candidate region gen 2ENSMUSG00000080476SNORA445.650.00Hypothetical protein PNA5 123ENSMUSG0000006807SNORA1712.140.00Hypothetical protein PNA5 123ENSMUSG000006852SNORA25-0.880.04Poly(A) binding protein, cytoplasmic 4ENSMUSG0000064555SNORA55-0.880.04Poly(A) binding protein, cytoplasmic 4ENSMUSG0000064558SNORA55-0.880.04Poly(A) binding protein, cytoplasmic 4ENSMUSG0000064558SNORA55-0.880.04Lnc-NA imprinted and accumulated in nucleusSham vs. DIM jointENSMUSG000006458SNORA43-1.120.04Lnc-NA imprinted and accumulated in nucleusENSMUSG0000066105SNORA43-1.120.04Small nuclear ribonucleoprotein NENSMUSG0000066105SNORA64-1.620.04Small nuclear ribonucleoprotein NENSMUSG000006611SNORD11610.560.01Predicted geneENSMUSG0000066493miR30683.310.03AlkB, alkylation repair homolog 1ENSMUSG0000064918SNORD182.210.01Ribosomal protein 1.5ENSMUSG0000064918SNORD44-1.030.05Ribusomal protein 1.4ENSMUSG0000064914SNORD46-1.060.04Ribosomal protein 1.17EN		ENSMUSG0000065353	SNORD73B	2.79	0.00	Regulator of chromosome condensation 1 and an lnc
ENSMUSG0000065778SNORA553.100.02Poly(A) binding protein, cytoplasmic 4ENSMUSG0000080478SNORD234.420.00Glioma tumor suppressor candidate region gene 2ENSMUSG0000080478SNORA445.650.00Hypothetical protein PNA5-123ENSMUSG00000688040SNORA1712.140.00Hypothetical protein MCG (307, homology human collagen alpha 2ENSMUSG0000068552SNORA213.400.00Hypothetical protein, cytoplasmic 4ENSMUSG0000064553SNORA55-0.880.04Poly(A) binding protein, cytoplasmic 4ENSMUSG0000064553SNORA43-1.120.04Lnc-Na imprinted and accumulated in nucleusSham vs. DMM jointENSMUSG0000064553SNORA43-1.120.04T-complex protein 1ENSMUSG0000064553SNORA44-1.620.04Small nucleolar RNA host gene 7ENSMUSG0000064510SNORA161.620.04Small nucleolar RNA host gene 7ENSMUSG0000064515SNORA64-1.620.04Small nucleolar RNA host gene 7ENSMUSG0000064516SNORA64-1.620.04Small nucleolar RNA host gene 7ENSMUSG0000064518SNORD11610.560.01Predicted geneENSMUSG0000064518SNORA64-1.620.04Small nucleolar RNA host gene 7ENSMUSG0000064518SNORD182.210.01Ribosomal protein L1ENSMUSG0000064518SNORA641.850.04Ribosomal protein L4ENSMUSG000006574SNORA641.850.04Ribosomal protein L17 <td>ENSMUSG0000064696</td> <td>SNORD20</td> <td>3.09</td> <td>0.00</td> <td>Nucleolin</td>		ENSMUSG0000064696	SNORD20	3.09	0.00	Nucleolin
ENSMUSG0000080478SNORD234.420.00Glioma tumor suppressor candidate region gene 2ENSMUSG0000095176SNORA445.650.00Hypothetical protein MGC16037, homology human collagen alpha 2ENSMUSG0000068804SNORA1712.140.00Hypothetical protein MGC16037, homology human collagen alpha 2ENSMUSG0000068552SNORA213.400.00Hypothetical protein, cytoplasmic 4ENSMUSG0000064655SNORA55-0.880.04Poly(A) binding protein, cytoplasmic 4ENSMUSG0000064658SNORA13-1.120.04Lnc-Na imprinted and accumulated in nucleusSham vs. DMM jointiENSMUSG000006455SNORA29-1.220.04T-complex protein 1ENSMUSG00000064505SNORA29-1.220.04Small nucleolar RNA host gene 7ENSMUSG00000065105SNORA64-1.620.04Small nucleor ribonucleoprotein NENSMUSG00000065105SNORA164-1.620.04Ensmulceor ribonucleoprotein NENSMUSG0000006517SNORD11610.560.01Predicted geneENSMUSG00000064918SNORD182.210.01Ribosomal protein L5ENSMUSG0000006524SNORD49-1.030.05RIKEN cDNA 241006H16 geneENSMUSG000006534SNORD49-1.030.05RIKEN cDNA 241006H16 geneENSMUSG000006534SNORD28-1.180.01Ribosomal protein L17ENSMUSG000006534SNORD28-1.180.01Ribosomal protein L17ENSMUSG000006453SNORD28-1.180.01Ribosomal		ENSMUSG0000065778	SNORA55	3.10	0.02	Poly(A) binding protein, cytoplasmic 4
ENSMUSG0000095176SNORA445.650.00Hypothetical protein PNAS-123ENSMUSG0000088040SNORA1712.140.00Hypothetical protein MGC16037, homology human colagen alpha 2ENSMUSG0000065852SNORA213.400.00Hypothetical protein, cytoplasmic 4ENSMUSG0000066455SNORA55-0.880.04Poly(A) binding protein, cytoplasmic 4ENSMUSG0000064858SNORA43-1.120.04Lnc-NA imprinted and accumulated in nucleusENSMUSG0000064515SNORA29-1.220.04T-complex protein 1ENSMUSG00000065105SNORA29-1.220.04Small nucleolar RNA host gene 7ENSMUSG00000065105SNORA64-1.620.04Small nucleolar ribonucleoprotein NENSMUSG0000009017SNORA64-1.620.04Small nucleolar ribonucleoprotein NENSMUSG0000006917SNORA643.310.03AlkB, alkylation repair homolog 1ENSMUSG000006918SNORA363.050.01DyskerinENSMUSG000006919SNORA662.780.05Ribosomal protein L5ENSMUSG0000064918SNORA641.850.04Small nuclear ribonucleoprotein NENSMUSG0000064917U901.900.01ImportinENSMUSG0000065734SNORD49A-1.030.05RIKEN cDNA 2410006H16 geneENSMUSG0000064545SNORD58-1.180.01Ribosomal protein L13ENSMUSG0000065734SNORD58-1.180.01Ribosomal protein L17ENSMUSG0000064571SNORD58-1.180.01<		ENSMUSG0000080478	SNORD23	4.42	0.00	Glioma tumor suppressor candidate region gene 2
ENSMUSG0000088040SNORA1712.140.00Hypothetical protein MGC16037, homology human collagen alpha 2ENSMUSG0000068552SNORA213.400.00Hypothetical protein, 152aaENSMUSG0000068555SNORA55-0.880.04Poly(A) binding protein, cytoplasmic 4ENSMUSG0000089715SNORA13-0.990.04Lnc-NA imprinted and accumulated in nucleusENSMUSG0000086505SNORA29-1.120.04T-complex protein 1ENSMUSG0000071711SNORA64-1.620.04Small nuclear ribonucleoprotein NENSMUSG0000064938miR30683.310.03AlkB, alkylation repair homolog 1ENSMUSG0000064931SNORA363.050.01DyskerinENSMUSG0000064931SNORA363.050.01DyskerinENSMUSG0000064931SNORA362.780.05Ribosomal protein L5ENSMUSG0000064931SNORA361.920.01ImportinENSMUSG0000064931SNORA641.850.04Small nuclear ribonucleoprotein NENSMUSG0000064931SNORA641.850.04Small nuclear ribonucleoprotein NENSMUSG0000064935SNORD68-1.100.01ImportinENSMUSG000006493SNORD18-1.220.01Ribosomal protein L13ENSMUSG000006493SNORD21-1.220.01Ribosomal protein L13ENSMUSG000006493SNORD58-1.180.01Ribosomal protein L17ENSMUSG000006493SNORD58-1.140.04Nucleosome assembly protein 1.16ENSM		ENSMUSG0000095176	SNORA44	5.65	0.00	Hypothetical protein PNAS-123
ENSMUSG0000065852SNORA213.400.00Hypothetical protein, 152aaENSMUSG0000064655SNORA55-0.880.04Poly(A) binding protein, cytoplasmic 4ENSMUSG0000064655SNORA55-0.880.04Inc-NA imprinted and accumulated in nucleusENSMUSG0000064658SNORA43-1.120.04Lnc-NA imprinted and accumulated in nucleusENSMUSG0000064858SNORA29-1.220.04Lnc-small nucleolar RNA host gene 7ENSMUSG00000077191SNORA64-1.620.04Small nuclear ribonucleoprotein NENSMUSG00000077191SNORA64-1.620.04Small nuclear ribonucleoprotein NENSMUSG00000064938miR30683.310.03AlkB, alkylation repair homolog 1ENSMUSG00000064938miR30683.050.01DyskerinENSMUSG0000064938SNORA363.050.01DyskerinENSMUSG0000064938SNORD182.210.01Ribosomal protein L5ENSMUSG0000064918SNORD182.210.01ImportinENSMUSG0000064400U31.920.01ImportinENSMUSG0000064400U31.920.01ImportinENSMUSG0000064440U31.920.01ImportinENSMUSG0000064574SNORD4A-1.800.04Ritken cDNA 241006H16 geneENSMUSG0000064444SNORD58-1.180.01Ritbosomal protein		ENSMUSG0000088040	SNORA17	12.14	0.00	Hypothetical protein MGC16037, homology human collagen alpha 2
ENSMUSG0000064655SNORA55-0.880.04Poly(A) binding protein, cytoplasmic 4ENSMUSG0000098971SNORD113-0.990.04Lnc-NA imprinted and accumulated in nucleusSham vs. DMM jointENSMUSG0000064858SNORA43-1.120.04Lnc- small nucleolar RNA host gene 7ENSMUSG00000065105SNORA29-1.220.04T-complex protein 1ENSMUSG00000077191SNORA64-1.620.04Small nuclear ribonucleoprotein NENSMUSG0000096017SNORD11610.560.01Predicted geneENSMUSG0000064938miR30683.310.03AlkB, alkylation repair homolog 1ENSMUSG0000064938SNORA363.050.01DyskerinENSMUSG0000064938SNORA662.780.05Ribosomal protein 1.5ENSMUSG0000064918SNORD182.210.01Ribosomal protein 1.4ENSMUSG0000064917U901.900.01ImportinENSMUSG0000064918SNORA641.850.04Small nuclear ribonucleoprotein NENSMUSG0000064919SNORA641.850.04Small nuclear ribonucleoprotein NENSMUSG0000064918SNORD14-1.030.05Riken cDNA 2410006H16 geneENSMUSG0000064934SNORD58-1.180.01Ribosomal protein 1.13ENSMUSG0000064450SNORD58-1.180.01Ribosomal protein 1.17ENSMUSG0000064451SNORD58-1.180.01Ribosomal protein 1.5ENSMUSG0000064451SNORD58-1.310.05Ribosomal protein 1.5		ENSMUSG0000065852	SNORA2	13.40	0.00	Hypothetical protein, 152aa
ENSMUSG0000098971 SNORD113 -0.99 0.04 Lnc-NA imprinted and accumulated in nucleus Sham vs. DMM joint ENSMUSG0000064858 SNORA43 -1.12 0.04 Lnc-NA imprinted and accumulated in nucleus ENSMUSG0000064505 SNORA29 -1.22 0.04 T-complex protein 1 ENSMUSG0000007191 SNORA64 -1.62 0.04 Small nuclear ribonucleoprotein N ENSMUSG000006417 SNORD116 10.56 0.01 Predicted gene ENSMUSG00000064938 miR3068 3.31 0.03 AlkB, alkylation repair homolog 1 ENSMUSG0000007222 SNORA66 2.78 0.05 Ribosomal protein L5 ENSMUSG00000064918 SNORD18 2.21 0.01 Ribosomal protein L4 ENSMUSG00000064918 SNORD43 -1.03 0.05 RIKEN cDNA 2410006H16 gene ENSMUSG00000067709 SNORA64 1.85 0.04 Small nuclear ribonucleoprotein N ENSMUSG00000064450 SNORD68 -1.06 0.04 Ribosomal protein L13 ENSMUSG00000064450 SNORD58 -1.18 0.01 Ribosomal pr	Sham vs. DMM joint	ENSMUSG0000064655	SNORA55	-0.88	0.04	Poly(A) binding protein, cytoplasmic 4
Sham vs. DMM jointENSMUSG0000064858SNORA43-1.120.04Lnc- small nucleolar RNA host gene 7ENSMUSG0000005105SNORA29-1.220.04T-complex protein 1ENSMUSG0000007191SNORA64-1.620.04Small nuclear ribonucleoprotein NENSMUSG0000006107SNORD11610.560.01Predicted geneENSMUSG00000064938miR30683.310.03AlkB, alkylation repair homolog 1ENSMUSG00000000017SNORA662.780.05Ribosomal protein L5ENSMUSG00000064918SNORA662.780.01Predicted geneENSMUSG00000064918SNORD182.210.01Ribosomal protein L4ENSMUSG00000064900U31.920.01ImportinENSMUSG00000064917U901.900.01ImportinENSMUSG00000077209SNORA641.850.04Small nuclear ribonucleoprotein NENSMUSG0000006450SNORD88-1.060.04Ribosomal protein L13ENSMUSG0000006453SNORD21-1.220.01Ribosomal protein L13ENSMUSG0000064543SNORD27-1.310.01Small nuclear RNA host gene 1ENSMUSG0000064513SNORD27-1.310.01Ribosomal protein L17ENSMUSG0000064513SNORD27-1.310.01Small nuclear RNA host gene 1ENSMUSG0000064571SNORD46-1.460.01Ribosomal protein L17ENSMUSG0000007756SNORD48-1.410.04Nucleosome assembly protein 1-like 1ENSMUSG00000077756SN		ENSMUSG0000098971	SNORD113	-0.99	0.04	Lnc-NA imprinted and accumulated in nucleus
ENSMUSG0000065105SNORA29-1.220.04T-complex protein 1ENSMUSG0000077191SNORA64-1.620.04Small nuclear ribonucleoprotein NENSMUSG000006017SNORD11610.560.01Predicted geneENSMUSG0000064938miR30683.310.03AlkB, alkylation repair homolog 1ENSMUSG0000089014SNORA363.050.01DyskerinENSMUSG000007222SNORA662.780.05Ribosomal protein L5ENSMUSG0000064918SNORD182.210.01Ribosomal protein L4ENSMUSG0000064400U31.920.01ImportinENSMUSG0000064400U31.920.01ImportinENSMUSG0000064400U31.920.01ImportinENSMUSG0000064400U31.920.01ImportinENSMUSG0000064400U31.920.01ImportinENSMUSG0000064400U31.920.01ImportinENSMUSG0000064400U31.920.01ImportinENSMUSG000006444SNORD68-1.060.04Ribosomal protein L1ENSMUSG000006444SNORD58-1.180.01Ribosomal protein L13ENSMUSG0000064453SNORD21-1.220.01Ribosomal protein L5ENSMUSG0000064841SNORD58B-1.400.05Ribosomal protein L17ENSMUSG000006781SNORD58B-1.400.05Ribosomal protein 1-like 1ENSMUSG0000007776SNORD46-1.460.01Ribosomal protein S8ENSMUSG		ENSMUSG0000064858	SNORA43	-1.12	0.04	Lnc- small nucleolar RNA host gene 7
ENSMUSG0000077191 SNORA64 -1.62 0.04 Small nuclear ribonucleoprotein N ENSMUSG0000096017 SNORD116 10.56 0.01 Predicted gene ENSMUSG00000064938 miR3068 3.31 0.03 AlkB, alkylation repair homolog 1 ENSMUSG00000064938 miR3068 3.05 0.01 Dyskerin ENSMUSG0000007222 SNORA66 2.78 0.05 Ribosomal protein L5 ENSMUSG00000064918 SNORD18 2.21 0.01 Predicted gene ENSMUSG00000064918 SNORD18 2.21 0.01 Importin L4 ENSMUSG00000064917 U90 1.90 0.01 Importin ENSMUSG0000007709 SNORA64 1.85 0.04 Small nuclear ribonucleoprotein N ENSMUSG00000065734 SNORD49A -1.03 0.05 RIKEN cDNA 2410006H16 gene ENSMUSG00000064574 SNORD68 -1.06 0.04 Ribosomal protein L17 ENSMUSG0000006453 SNORD21 -1.22 0.01 Ribosomal protein L17 ENSMUSG00000064844 SNORD27 -1.31		ENSMUSG0000065105	SNORA29	-1.22	0.04	T-complex protein 1
ENSMUSG0000096017SNORD11610.560.01Predicted geneENSMUSG0000064938miR30683.310.03AlkB, alkylation repair homolog 1ENSMUSG00000089014SNORA363.050.01DyskerinENSMUSG0000077222SNORA662.780.05Ribosomal protein L5ENSMUSG0000064918SNORD182.210.01Ribosomal protein L4ENSMUSG000006490U31.920.01Predicted geneENSMUSG000006490U31.920.01ImportinENSMUSG000006490U31.920.01ImportinENSMUSG000006490U31.920.01ImportinENSMUSG000006490U31.920.01ImportinENSMUSG000006490SNORA641.850.04Small nuclear ribonucleoprotein NENSMUSG0000065734SNORD49A-1.030.05RIKEN cDNA 2410006H16 geneENSMUSG000006450SNORD58-1.180.01Ribosomal protein L13ENSMUSG000006453SNORD21-1.220.01Ribosomal protein L5ENSMUSG000006453SNORD27-1.310.01Small nucleolar RNA host gene 1ENSMUSG0000006451SNORD58B-1.400.05Ribosomal protein L17ENSMUSG0000006451SNORD46-1.460.01Ribosomal protein 14ENSMUSG00000067716SNORD46-1.460.01Ribosomal protein 14ENSMUSG00000067756SNORD90-1.480.01Ribosomal protein 88ENSMUSG00000067756SNORD90-1.480.03<		ENSMUSG0000077191	SNORA64	-1.62	0.04	Small nuclear ribonucleoprotein N
ENSMUSG0000064938 miR3068 3.31 0.03 AlkB, alkylation repair homolog 1 ENSMUSG00000089014 SNORA36 3.05 0.01 Dyskerin ENSMUSG00000077222 SNORA66 2.78 0.05 Ribosomal protein L5 ENSMUSG0000064918 SNORD18 2.21 0.01 Ribosomal protein L4 ENSMUSG00000064400 U3 1.92 0.01 Predicted gene ENSMUSG00000089417 U90 1.90 0.01 Importin ENSMUSG0000007729 SNORA64 1.85 0.04 Small nuclear ribonucleoprotein N ENSMUSG00000065734 SNORD49A -1.03 0.05 RIKEN cDNA 2410006H16 gene ENSMUSG0000006450 SNORD68 -1.06 0.04 Ribosomal protein L13 ENSMUSG00000064450 SNORD58 -1.18 0.01 Ribosomal protein L17 ENSMUSG00000064844 SNORD27 -1.31 0.01 Small nucleolar RNA host gene 1 ENSMUSG00000064871 SNORD58B -1.40 0.05 Ribosomal protein L17 ENSMUSG00000064871 SNORD46 -1.41 <td></td> <td>ENSMUSG0000096017</td> <td>SNORD116</td> <td>10.56</td> <td>0.01</td> <td>Predicted gene</td>		ENSMUSG0000096017	SNORD116	10.56	0.01	Predicted gene
ENSMUSG0000089014 SNORA36 3.05 0.01 Dyskerin ENSMUSG0000077222 SNORA66 2.78 0.05 Ribosomal protein L5 ENSMUSG0000064918 SNORD18 2.21 0.01 Ribosomal protein L4 ENSMUSG0000064400 U3 1.92 0.01 Predicted gene ENSMUSG00000089417 U90 1.90 0.01 Importin ENSMUSG0000000077709 SNORA64 1.85 0.04 Small nuclear ribonucleoprotein N ENSMUSG0000065734 SNORD49A -1.03 0.05 RIKEN cDNA 2410006H16 gene ENSMUSG0000006450 SNORD68 -1.06 0.04 Ribosomal protein L13 ENSMUSG00000064450 SNORD58 -1.18 0.01 Ribosomal protein L17 ENSMUSG00000064453 SNORD21 -1.22 0.01 Ribosomal protein L5 ENSMUSG00000064871 SNORD58B -1.40 0.05 Ribosomal protein L17 ENSMUSG00000064751 SNORD46 -1.46 0.01 Ribosomal protein L17 ENSMUSG00000064751 SNORD46 -1.46 0.01 <td rowspan="17">Sham vs. DMM serum</td> <td>ENSMUSG0000064938</td> <td>miR3068</td> <td>3.31</td> <td>0.03</td> <td>AlkB, alkylation repair homolog 1</td>	Sham vs. DMM serum	ENSMUSG0000064938	miR3068	3.31	0.03	AlkB, alkylation repair homolog 1
ENSMUSG00000077222SNORA662.780.05Ribosomal protein L5ENSMUSG0000064918SNORD182.210.01Ribosomal protein L4ENSMUSG0000064400U31.920.01Predicted geneENSMUSG0000089417U901.900.01ImportinENSMUSG00000077709SNORA641.850.04Small nuclear ribonucleoprotein NENSMUSG0000065734SNORD49A-1.030.05RIKEN cDNA 2410006H16 geneENSMUSG0000064450SNORD68-1.060.04Ribosomal protein L13ENSMUSG0000064453SNORD58-1.180.01Ribosomal protein L17ENSMUSG0000064453SNORD21-1.220.01Ribosomal protein L5ENSMUSG0000064871SNORD58-1.310.01Small nucleolar RNA host gene 1ENSMUSG0000064751SNORD46-1.400.05Ribosomal protein L17ENSMUSG0000064751SNORD46-1.460.01Ribosomal protein 1-like 1ENSMUSG0000064751SNORD46-1.460.01Ribosomal protein S8ENSMUSG0000064751SNORD90-1.480.01Ring finger and CCCH-type zinc finger domains 2		ENSMUSG0000089014	SNORA36	3.05	0.01	Dyskerin
ENSMUSG0000064918SNORD182.210.01Ribosomal protein L4ENSMUSG0000064400U31.920.01Predicted geneENSMUSG0000089417U901.900.01ImportinENSMUSG0000077709SNORA641.850.04Small nuclear ribonucleoprotein NENSMUSG0000065734SNORD49A-1.030.05RIKEN cDNA 2410006H16 geneENSMUSG0000064450SNORD68-1.060.04Ribosomal protein L13ENSMUSG0000064453SNORD58-1.180.01Ribosomal protein L17ENSMUSG0000064453SNORD21-1.220.01Ribosomal protein L5ENSMUSG0000064451SNORD27-1.310.01Small nucleolar RNA host gene 1ENSMUSG0000064871SNORD58B-1.400.05Ribosomal protein L17ENSMUSG0000064751SNORD46-1.460.01Ribosomal protein 1-like 1ENSMUSG0000064751SNORD46-1.460.01Ribosomal protein S8ENSMUSG00000077756SNORD90-1.480.01Ring finger and CCCH-type zinc finger domains 2ENSMUSG00000087913SNORD11-1.860.03NOP58 ribourcleoprotein S8		ENSMUSG0000077222	SNORA66	2.78	0.05	Ribosomal protein L5
ENSMUSG0000064400U31.920.01Predicted geneENSMUSG0000064400U31.900.01ImportinENSMUSG0000089417U901.900.01ImportinENSMUSG0000077709SNORA641.850.04Small nuclear ribonucleoprotein NENSMUSG0000065734SNORD49A-1.030.05RIKEN cDNA 2410006H16 geneENSMUSG0000064450SNORD68-1.060.04Ribosomal protein L13ENSMUSG0000064453SNORD58-1.180.01Ribosomal protein L5ENSMUSG0000064531SNORD21-1.220.01Ribosomal protein L5ENSMUSG0000064871SNORD58B-1.400.05Ribosomal protein L17ENSMUSG0000064751SNORD464-1.410.04Nucleosome assembly protein 1-like 1ENSMUSG0000064751SNORD46-1.460.01Ribosomal protein S8ENSMUSG0000064751SNORD90-1.480.01Ring finger and CCCH-type zinc finger domains 2ENSMUSG00000087093SNORD11-1.860.03NOP58 ribonucleareretein		ENSMUSG0000064918	SNORD18	2.21	0.01	Ribosomal protein L4
ENSMUSG0000089417U901.900.01ImportinENSMUSG0000077709SNORA641.850.04Small nuclear ribonucleoprotein NENSMUSG0000065734SNORD49A-1.030.05RIKEN cDNA 2410006H16 geneENSMUSG0000064500SNORD68-1.060.04Ribosomal protein L13ENSMUSG0000064450SNORD68-1.180.01Ribosomal protein L13ENSMUSG0000064453SNORD21-1.220.01Ribosomal protein L5ENSMUSG0000064531SNORD27-1.310.01Small nucleolar RNA host gene 1ENSMUSG0000064871SNORD58B-1.400.05Ribosomal protein L17ENSMUSG0000064751SNORD46-1.410.04Nucleosome assembly protein 1-like 1ENSMUSG0000064751SNORD46-1.460.01Ribosomal protein S8ENSMUSG00000077756SNORD90-1.480.01Ring finger and CCCH-type zinc finger domains 2ENSMUSG00000089093SNORD11-1.860.03NOP58 ribonucleoprotein		ENSMUSG0000064400	U3	1.92	0.01	Predicted gene
Sham vs. DMM serumENSMUSG00000077709SNORA641.850.04Small nuclear ribonucleoprotein NENSMUSG0000065734SNORD49A-1.030.05RIKEN cDNA 2410006H16 geneENSMUSG0000064450SNORD68-1.060.04Ribosomal protein L13ENSMUSG0000064453SNORD58-1.180.01Ribosomal protein L17ENSMUSG0000064453SNORD21-1.220.01Ribosomal protein L5ENSMUSG0000064871SNORD27-1.310.01Small nucleolar RNA host gene 1ENSMUSG0000064871SNORD58B-1.400.05Ribosomal protein L17ENSMUSG0000064751SNORD46-1.410.04Nucleosome assembly protein 1-like 1ENSMUSG0000064751SNORD46-1.460.01Ribosomal protein S8ENSMUSG00000077756SNORD90-1.480.01Ring finger and CCCH-type zinc finger domains 2ENSMUSG00000087093SNORD11-1.860.03NOP58 ribonucleoperatein		ENSMUSG0000089417	U90	1.90	0.01	Importin
Sham vs. DMM serum ENSMUSG0000065734 SNORD49A -1.03 0.05 RIKEN cDNA 2410006H16 gene ENSMUSG00000654450 SNORD68 -1.06 0.04 Ribosomal protein L13 ENSMUSG0000064450 SNORD58 -1.18 0.01 Ribosomal protein L17 ENSMUSG0000064453 SNORD21 -1.22 0.01 Ribosomal protein L5 ENSMUSG0000066453 SNORD27 -1.31 0.01 Small nucleolar RNA host gene 1 ENSMUSG00000664871 SNORD58B -1.40 0.05 Ribosomal protein L17 ENSMUSG00000664751 SNORD68B -1.40 0.05 Ribosomal protein L17 ENSMUSG0000064751 SNORD58B -1.40 0.05 Ribosomal protein L17 ENSMUSG0000064751 SNORD46 -1.40 0.04 Nucleosome assembly protein 1-like 1 ENSMUSG00000067756 SNORD46 -1.46 0.01 Ribosomal protein S8 ENSMUSG00000077756 SNORD90 -1.48 0.01 Ring finger and CCCH-type zinc finger domains 2 ENSMUSG00000087093 SNORD11 -1.86 0.03 NOP58 ribouncleoparctein<		ENSMUSG0000077709	SNORA64	1.85	0.04	Small nuclear ribonucleoprotein N
Sham vs. DMM serum Image: Constraint of the serue of the		ENSMUSG0000065734	SNORD49A	-1.03	0.05	RIKEN cDNA 2410006H16 gene
ENSMUSG0000064844SNORD58-1.180.01Ribosomal protein L17ENSMUSG0000064843SNORD21-1.220.01Ribosomal protein L5ENSMUSG0000065281SNORD27-1.310.01Small nucleolar RNA host gene 1ENSMUSG0000064871SNORD58B-1.400.05Ribosomal protein L17ENSMUSG00000064871SNORD58B-1.400.05Ribosomal protein 1.17ENSMUSG00000064751SNORD48-1.410.04Nucleosome assembly protein 1-like 1ENSMUSG00000064751SNORD46-1.460.01Ribosomal protein S8ENSMUSG00000077756SNORD90-1.480.01Ring finger and CCCH-type zinc finger domains 2ENSMUSG00000089093SNORD11-1.860.03NOP58 ribonucleoparatein		ENSMUSG0000064450	SNORD68	-1.06	0.04	Ribosomal protein L13
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ENSMUSG00000064871SNORD58B-1.400.05Ribosomal protein L17ENSMUSG00000087819SNORA48-1.410.04Nucleosome assembly protein 1-like 1ENSMUSG00000064751SNORD46-1.460.01Ribosomal protein S8ENSMUSG00000077756SNORD90-1.480.01Ring finger and CCCH-type zinc finger domains 2ENSMUSG00000087903SNORD11-1.860.03NOP58 ribourcleoprotein		ENSMUSG0000065281	SNORD27	-1.31	0.01	Small nucleolar RNA host gene 1
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ENSMUSG0000007775 SNORD46 -1.46 0.01 Ribosomal protein S8 ENSMUSG00000077756 SNORD90 -1.48 0.01 Ring finger and CCCH-type zinc finger domains 2 ENSMUSG00000089093 SNORD11 -1.86 0.03 NOP58 ribourcleoprotein		ENSMUSG0000004871	SNOR 448	-1.40	0.03	Nucleosome assembly protein 1-like 1
ENSMUSG00000004731 SNORD40 -1.40 0.01 Ribdsonial protein 38 ENSMUSG00000077756 SNORD90 -1.48 0.01 Ring finger and CCCH-type zinc finger domains 2 ENSMUSG00000089093 SNORD11 -1.86 0.03 NOP58 ribonucleoprotein		ENSMUSC00000064751	SNODD44	_1.41	0.04	Ribosomal protain Co
EXISTRUSSCO00000077750 SINCKD90 -1.46 0.01 King inger and CCCH-type Zinc inger domains 2 ENSMUSC00000089093 SNORD11 -1.86 0.03 NOP58 ribonucleoprotein		ENSMUSC0000004/51	SNOPD00	-1.40	0.01	Ping finger and CCCU time sing finger demoise 2
		ENSMUSG0000077756	SNORD11	-1.40	0.01	NOP58 ribonucleoprotein

Table 2. Differentially expressed snoRNAs between contrasts including host gene identification.Explanation: positive $Log_2 FC =$ increased in old or DMM. Negative $Log_2 FC =$ is decreased in old or DMM.

was significantly decreased in young versus old joint (Fig. 3A), while SNORA73 was validated to be increased in young versus old joint (Fig. 3A). In agreement with its absence in the DE group (Table 2), SNORA30 was not differentially expressed in young versus old joint (Fig. 3A) and SNORD88 was not differentially expressed in sham versus DMM joint (Fig. 3A). SNORA31, SNORA28, SNORD23 and SNORA73 were confirmed to be significantly increased in young versus old serum (Fig. 3B) and SNORD116, SNORA64 and U3 were significantly increased in sham versus DMM serum (Fig. 3C). SNORD46 was confirmed to be significantly decreased in DMM serum as compared to sham serum (Fig. 3C).

SNORD116 in equine serum in OA. To confirm increased SNORD116 in OA serum levels in a different species using qRT-PCR, we measured SNORD116 in equine serum samples. Normal equine donors had a Mankin's score 1.25 ± 0.9 (mean $\pm 95\%$ CI) and OA donors 7.75 ± 7.6 . Synovial membrane from normal donors had a synovitis score²⁷ of 3.25 ± 2.3 and OA donors 3.25 ± 3.2 . There was a significant increase in serum expression of SNORD116 in OA compared to normal donors (p = 0.0010) (Fig. 4).

Discussion

SnoRNAs are emerging as important regulators of cell functions, such as alternative splicing²⁸, metabolic stress²⁹ and development of disease; in cancer⁶, Prader-Willi Syndrome and autism⁵. Through expression profiling of snoRNAs using deep-sequencing we reveal novel molecular features relating to joint ageing and OA.

Whilst gene expression has been evaluated in animal models of OA, including the rat anterior cruciate transection³⁰ and meniscal tear models³¹, and mouse DMM model³² these were primarily interrogating protein-coding genes. Furthermore, apart from the final study these experiments evaluated a single tissue, primarily articular cartilage. In musculoskeletal ageing single tissues have been investigated^{13,14}. OA is a process that involves the whole joint as an organ; therefore we undertook our analysis on whole mouse joints, which included cartilage, meniscus, subchondral bone, and joint capsule with synovium.

Mice are considered skeletally mature at around 3-months-old (approximate equivalent of a teenaged human) while a 12-month-old mouse would signify a 40 to 50 year human³³. Thus, to investigate the effects of joint age we used 8-month-old equivalent to 25 to 28 year human (referred to as young) and 24-month-old mice (referred to as old). To study the development of post-traumatic OA we measured OA severity histologically and analysed snoRNAs expression in joints and serum from 24-month-old mice following DMM. Limitations of this study were that we did not additionally undertake the DMM model in young mice. Additionally the 'old' age in young versus old contrast was not the same age as 'old' age in sham versus DMM. OARSI scores that were observed in the sham group (DMM surgery cohort; 24 months old) were lower than the old group (young versus old cohort; 18 months old). At this moment we cannot explain this difference, but different housing and environments of the two cohorts may have influenced this. The DMM model is a post-injury model in which the histological lesions within the affected joint are similar to those observed in human OA¹⁷. Mild OA-like pathology was present in the old and sham mice implying that mice at this age are in the early stages of acquiring naturally occurring OA comparable to studies of human knees at the equivalent age of approximately 40 years-old³⁴. However the effect of the DMM model exacerbated these changes.

Detection of snoRNAs in serum has previously been demonstrated^{12,35,36}. SnoRNAs are serum stable, and although normally resident in the nucleolus it is thought in serum they are present as unidentified protein complexes¹². It is however not clear whether disease-associated RNAs detected in the circulation result from local tissue disturbances and cell death, or whether they are actively locally secreted via exosomes or microvesicles or are a systemic response upon local tissue damage^{8,37,38}. This may even depend on the specific pathology or specific RNA species. Determining the average read-length distribution of representative joint and serum samples (Fig. 2C) revealed an unexpected finding. When looking at the read-length distribution the serum reads specifically contain a large peak of RNAs with a length of approximately 30 nt. The combined realisation that quite a large portion of the reads could not be mapped back to the used genome database, that a large portion of the piRNA class of non-coding RNAs lacks annotation in the used genome database and that piRNAs are typically 30 nt long, makes it is reasonable to think that this explains the presence of the large 30 nt peak specifically in serum. Only recently the presence of piRNAs in human blood has been described³⁹ and this significant difference in the constitution of small RNAs for joint and serum samples is a phenomenon calling for further investigation.

In the young versus old serum (27 snoRNAs) more snoRNAs were significantly up-regulated compared to the old sham versus old DMM serum (18 snoRNAs) indicating ageing *per se* had a greater effect on differential snoRNA presence in serum than OA. This could be due to different 'old' ages in the comparisons, but also maybe expected as in ageing serum snoRNAs will be from many tissues whereas in the DMM model we are most likely highlighting primarily OA joint-related snoRNAs. As we were studying the whole joint this may be due to varying expression of snoRNAs in joint tissues, as it is known that there is tissue-specific snoRNAs expression⁴⁰. We are unable to determine the amount each tissue type contributed to overall expression of snoRNAs. Each tissue will vary in its cellularity and hence RNA and snoRNAs content. Thus whilst a novel aspect of this study was that snoRNAs were extracted from the multiple tissues that form the joint, our approach may be less sensitive in detecting snoRNAs that change in a single tissue. However it has the advantage of determining snoRNAs that could be more globally implicated in OA. Despite the potential limitations, we identified a number of potentially interesting snoRNAs for future studies.

SNORA73 was increased in old joint and serum (Table 2, Fig. 3) and represents a potential joint 'biological ageing' marker. A reliable measurement of the state of ageing and a prediction of the risk of the onset of morbidity for chronic age-related diseases such as OA would be beneficial. Such a strategy could serve as a measure of 'biological' age and predict an age-related biological response more accurately than chronological age. SNORA64 was increased and SNORD46 was decreased in DMM serum, but both were not DE in young versus old serum,



Figure 3. SnoRNA expression from SnoRNASeq was validated with qRT-PCR. (**A**) Gene expression patterns of SNORD88, SNORA73 and SNORA30 were confirmed in young and old joint. SNORD88 expression was verified in sham and DMM joint. (**B**) SNORA31, SNORA28, SNORD23 and SNORA73 were confirmed to be increased in old serum. (**C**) Gene expression patterns of SNORD116, SNORA64, U3 and SNORD46 were validated in sham and DMM serum. Gene expression is depicted as fold induction relative to control (i.e. young or sham). Data represents the mean + 95% CI. For statistical evaluation an independent samples t-test was performed using GraphPad Prism 5 (San Diego) on log-transformed data; p-values are indicated.



Figure 4. Increased gene expression of SNORD116 in equine OA serum as compared to serum from normal donors. Data represents the mean + 95% CI. For statistical evaluation an independent samples t-test was performed using GraphPad Prism 5 (San Diego) on log-transformed data; p = 0.0010.

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indicating these snoRNAs as possible OA markers. SNORD18 was increased in serum both in ageing and following DMM (Table 2) signifying that they are affected in ageing and also OA. It is difficult to speculate how much this snoRNA changes in age-related OA versus age from this study. Histology identified mild increase in OARSI score with age and the level of OA changes in the DMM model was mild. It would be beneficial to investigate this snoRNAs further in tissues and serum in more severe OA.

An interesting finding was an increase in SNORD38 in ageing joint. Zhang *et al.*¹² demonstrated a strong association between serum levels of SNORD38 and severe cartilage damage, in anterior cruciate ligament (ACL) injury, enabling distinction between ACL injury patients from normal donors. They were unable to determine

age effect of SNORD38 in serum from normal donors as it was undetectable. One year post-surgery there was no relationship between donor age and serum SNORD38. Our study demonstrated an increase in SNORD38 in mouse joints (but not serum) with age. The human study did not assess tissue snoRNAs and the serum samples were primarily from middle-aged donors. Our old mice group represents an equivalent older age than that in the human study, which could contribute to this disparity.

The most DE snoRNA in DMM serum was SNORD116. Additionally we demonstrated an increase in serum SNORD116 in horses with MCP OA. We have previously identified SNORD116 as increased in OA compared to normal human cartilage in an array study¹⁵. A loss of SNORD116 is a significant contribution to the aetiology of the neurodegenerative genetic condition Prader-Willi syndrome (PWS)⁴¹. This paternally imprinted disorder results in developmental delay and genetic obesity due to hyperphagia⁴². Clinical signs include short stature and low bone mineral density⁴³. In a recent mouse transgenic study the loss of PWS critical region (including SNORD116) resulted in reduced bone mineral density (BMD), delayed skeletal development and reduced bone size and osteoblastic suppression⁴⁴. Humans with OA have an increased BMD in affected joints⁴⁵. Thus our findings not only elucidate a potential marker of OA but a snoRNA with a potential role in the pathogenesis of OA.

Previously we have identified DE snoRNAs in ageing cartilage¹³ and tendon¹⁴ and together with results in this study we propose that in musculoskeletal tissues snoRNAs potentially modulate the ageing process as previously described⁴⁶. While determining the transcriptomic signature of ageing equine cartilage¹³ we found the differential expression of a number of snoRNAs associated with ageing. When comparing the differentially expressed snoRNAs between the equine and this mouse study it is important to realize that in our previous equine study we did not analyse serum or the whole joint, but specifically the articular cartilage. Overlapping differential snoRNA expression between studies was identified for SNORD113, SNORA53, SNORA48 and SNORA5. SNORA53 was decreased in old equine cartilage, but increased in old mouse serum. SNORA48 was decreased in old equine cartilage, and decreased in old DMM mouse serum. SNORA5 was increased in old equine cartilage and increased in old mouse serum. Possibly the best consistency was found for SNORD113, which was decreased in old mouse joint (and old DMM mouse joint) and equine cartilage. The apparent cross-species conservations of differentially expressed snoRNAs in ageing and OA strengthen our belief that snoRNAs could indeed be used as biomarkers. Further analysis of snoRNA expression profiles and detailed genetic studies will give new insights into novel molecular networks in musculoskeletal ageing and common mechanisms in ageing and age-related diseases such as OA.

In mammalians the majority of snoRNAs are encoded within the introns of protein coding or non-coding genes; host-genes⁴⁷. There is evidence that genes which host snoRNAs might contribute to the aetiology of cancer through regulation of cell homeostasis and cancer biology (reviewed⁶). Potentially both the host-gene and the snoRNAs encoded within them may be important in different situations. For example growth-arrest-specific-5 (GAS-5) (hosts ten C/D box snoRNAs⁴⁸), a non-coding RNA which accumulates in growth arrested cells, regulates cell death and proliferation by acting as a decoy hormone response element for glucocorticoid receptors thereby inhibiting gene upregulation by activated glucocorticoid receptors⁴⁹. Interesting snoRNA host-genes were identified in this study including transforming growth factor β regulated gene 4, sorting nexin 5 and collagen type 1 (with roles in joint homeostasis). Therefore, an alteration of snoRNA expression may result from changes in transcriptional activity of the host-gene related to joint homeostasis or disease.

Conclusion

Our results implicate specific changes in snoRNA abundance in joint ageing (SNORD88 and SNORD38 were respectively decreased and increased) and OA suggesting the potential use of snoRNAs such as SNORA73 and SNORD23 as a novel biomarker for joint ageing, SNORA64, SNORD46 and SNORD116 for OA, SNORD18 for ageing and OA.

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Author Contributions

M.J.P., M.M.F.S.; conception and design, collection and assembly of data, data analysis and interpretation, manuscript writing, final approval of the manuscript. Y.F.; assembly of data, data analysis, manuscript writing, final approval of the manuscript. P.M.; provision of study material, assembly of data, manuscript writing and final approval of the manuscript. P.D.C., T.J.M.W., D.Y.; conception and design, manuscript writing, final approval of the manuscript.

Additional Information

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