In the C57BL/6 strain Sfrp1 is constantly expressed from 2 to 6 weeks, down-regulated thereafter and absent at 8 and 11 weeks of age. In the STR/orot strain, there is a more stable and fluctuating Sfrp1 expression over the time. In general the Sfrp1 expression appears to lower in 2 to 6 weeks old but higher in 8 and 11 weeks old STR/orot mice in comparison to C57BL/6 mice.

**Conclusions:** The identified OA-QTL contains the Sfrp1 gene which is involved in the Wnt signalling pathway regulating the differentiation of mesenchymal progenitor cells into osteoblasts and chondrocytes. Our sequencing results imply possible differences in the protein secretion, at alternative splicing and variable stability of the mRNA and include protein dysfunction as well as differential regulation. We do show expression differences of Sfrp1 in the knee joints of C57BL/6 and STR/orot strains which suggest an altered Wnt signalling. All in all, this supports the concept of OA resulting from disordered bone and cartilage differentiation processes.

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**GENE EXPRESSION PROFILE IN THE ARTICULAR CARTILAGE OF A NATURAL MODEL OF MOUSE OSTEOARTHRITIS**


**Purpose:** Susceptibility to osteoarthritis (OA) may be determined during different initiation and progression phases of this complex disease. To determine the factors contributing to each of these phases, we have mostly had to rely on animal models in which, unlike human OA, samples from all phases are available. The STR/orot mouse is an inbred strain that develops OA spontaneously with age, in which specific gene expression changes may take place in articular cartilage (AC) during the different phases of the disease. Our aim was to identify new candidates with pivotal roles in OA susceptibility. OA initiation and progression, by comparing gene expression profiles in articular cartilage (AC) from STR/orot mouse knees (with age-matched CBA mice as control) at various distinct, histologically-defined, stages of OA by microarray technology.

**Methods:** AC was isolated from left knees of 8-10, 18-20 and over 40 week-old male STR/orot and age-matched CBA mice using a micro-rongeur. Right knees were used for histological scoring of AC lesions. RNA was harvested in 4 independent replicates. RNA quality was assessed using the Agilent 2000 Bioanalyzer system and subsequently hybridized to Affymetrix© Mouse Gene 1.0 ST Arrays. After initial normalization, using the RNA algorithm and exon summarization, data were analyzed using the GeneSpring GX 11.1 analysis software and two-Way ANOVA, with a p value of <0.05 considered significant.

**Results:** Histological grading showed no differences in AC lesion severity at 8wks, but expected increases in STR/orot mice compared to CBA at both 18 and 40 wks of age. The data were then filtered on fold changes between different experimental groups and the number of genes significantly modified between the different groups were described in table 1. Overall, differences between strains were stronger than within one strain over time. With a fold change cut-off of 1.45 between STR/orot and CBA mice, 852 genes were differentially expressed at 8 weeks, 1530 at 18 weeks and 1131 in mice at 40 weeks of age. In addition, there were 116, 647 and 260 genes differentially regulated uniquely in 8, 18 and 40 week-old STR/orot mice, respectively. Using the probe sets >1.5 fold-change between CBA and STR/orot mice, and Ingenuity Pathway Analysis (IPA) software, we found that the main biological functions and canonical pathways affected were inflammation, immunological processes, skeletal, muscular and connective tissues (disorders, development and function); cell cycle (proliferation, death, cancer), cell signaling, metabolism and genetic disorders. Genes differentially expressed in STR/orot mouse AC at all ages, included: Mmp13, Mmp3, C10or1a1, Timp1 and Acan (Aggrecan); those uniquely differentially expressed at 8wks included Clip and Dkk1; at 18wks Mmp2, Timp2, Ugdh and Cgfl; at 40wks Col2a1, Psg4, Cox2, Il1a, Tgfb1, S100a8.

**Conclusions:** Our data show significant differences in AC gene expression in CBA and STR/orot mice at ages that represent diverse phases of natural OA, some of which have previously been shown to be modified during OA development. Studying specific gene changes will allow us to define new factors involved in susceptibility, initiation and progression in a model of spontaneous OA. Confirmation of gene expression changes between the CBA and STR/orot mice and with age by q-PCR and immunohistochemistry will help verify their authenticity as new targets.

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**COMBINING CHONDROCYTE GENE EXPRESSION, LITERATURE MINING AND PATHWAY/NETWORK ANALYSIS TO EXTRACT BIOLOGICAL INSIGHTS FROM SMALL-SCALE MICROARRAY DATA**

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**Purpose:** Prioritizing candidate genes for gene-disease associations based on experimental data is a common task in biomedical research. However, sample numbers per biological condition or treatment may be too small to obtain a reliable candidate ranking based on standard statistical methods. This is a limiting factor for interpreting data from high-throughput studies using microarrays. Here, we employ a new strategy combining expression analysis and gene name literature mining to rank genetic probes from small microarray datasets obtained from equine articular chondrocytes.

**Methods:** We used a two-step procedure to filter and rank candidate genes based on standard Affymetrix microarray data obtained from control cultures of primary equine articular chondrocytes and cultures exposed to interleukin 1-beta (IL-1β) and the anti-inflammatory plant derived phytochemical curcumin. First, genes with low variance across all samples were filtered out and the remaining genes were ranked using a statistical tool that accounts for the uncertainty within on-chip replicate data for each genetic probe. In the second step, we used text mining of the PubMed database based on a controlled vocabulary to compute a final combined ranking score for candidate genes. To enhance interpretability and increase robustness, we mapped top-ranked genes onto subcellular pathways and identified the pathways with largest changes in corresponding gene expression values.

**Results:** The 15 top-ranked genes identified included several genes already known to be associated with the inflammatory pathways affected by IL-1β and curcumin. Moreover, a network-topological analysis of the candidates mapped onto a comprehensive human protein-protein interaction network [4] to identify groups of genes with distinct topological properties in comparison to random genes, e.g. similar to known disease-associated genes they displayed a significantly higher connectivity (node degree) in the network than matched-size random gene sets. Similarly, the top-ranked de-regulated pathways had known associations with the biological conditions and the subcellular processes of interest. To rank the genes according to their association with Medical Subject Headings ("MeSH" terms) in the literature, the occurrences and co-occurrences of corresponding phrases in the PubMed database were counted to compute the ranking or Pointwise Mutual Information score (PMI score). The genes with significant PMI scores included protein phosphatase 1a (PPM1A), glutamate-cysteine ligase (GCLM), mitogen-activated protein kinase (MAPK32) and nuclear factor of kappa light chain gene enhancer (NFκBIA) with PMI scores of 5.91, 5.04, 5.02 and 4.6 respectively. Interestingly, the plasma membrane Na, K-ATPase was one of the genes that was found to be significantly differentially expressed between the control, IL-1β and curcumin treated chondrocyte cultures.

**Conclusions:** The results of the gene ranking, pathway and network analysis underscore that the combined gene ranking based on gene expression and literature mining can profitably be applied to small-scale microarray studies and experiments with small quantities of clinical material. The increased sensitivity achieved by combining gene expression and literature mining will facilitate data interpretation and enhanced prioritization of candidate genes for further validation using quantitative, real-time polymerase chain reaction studies and proteomics.