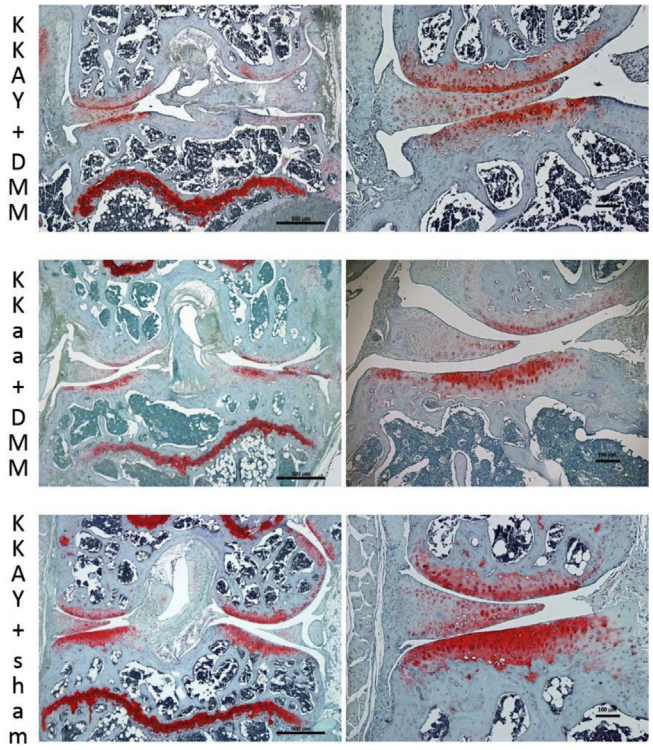
purpose of this study was to develop and use a new murine model to test the hypothesis that the presence of type 2 diabetes (without a high fat diet) worsens joint disease pathology.
Methods: The destabilization of the medial meniscus (DMM) model of OA was induced in a genetic mouse model of type 2 diabetes. The heterozygous KKA^{Y} mouse has polygenetic defects that result in hyperglycemia by eight weeks of age due primarily to insulin resistance. high fat diet which is commonly required of other models of diabetes. The DMM procedure appears to be successful in the male mice of this strain. However, at 8 weeks post-procedure, signs of joint damage are mild. Thus, it may be more useful to carry the experiment to 12 weeks post-procedure in order to achieve further progression of joint pathology and allow scoring of joints to distinguish differences between groups with and without diabetes.

Summary of OA prevalence, diabetes and body mass measures

Group	Mice with mild OA	Blood glucose mean (mg/dL)	Blood glucose range (mg/dL)	Body mass Mean (g)	Body mass range (g)
KKA^{Y} (diabetes + DMM)	66%	434	305–500+	41.1	38.5–43.5
KKaa (control + DMM)	33%	257	222–294	36.0	35.6–36.7
KKA^{Y} (diabetes + sham)	none	457	391–500+	38.2	35.8–39.8



The homozygous wild type KKaa siblings have normal blood sugar levels. Both KKA^{Y} and KKaa are overweight to moderately obese. The DMM procedure was performed on 12 week old male KKA^{Y} mice (N = 3) and KKaa mice (N = 3). A sham procedure was performed on separate 12 week old male KKA^{Y} mice (N = 3). All mice received normal chow diets and had normal cage activity until euthanasia which was 8 weeks post-procedure. Knee joints were obtained, fixed, decalcified, and thin-sectioned. Every third section was stained with safranin O, fast green, and iron hematoxylin. Sections were observed microscopically at low and high magnification for indications of joint degeneration including low or absent safranin O staining, cartilage fibrillation, chondrocyte cloning, early osteophyte formation, and tidemark duplication.
Results: The KKA^{Y} mice had significantly greater blood glucose levels ($P = 0.007$) but not significantly different body masses ($P = 0.054$) compare to the KKaa at 8 weeks post-procedure (Table). Two of the three KKA^{Y} mice had signs of mild OA, one of the three KKaa mice had signs of mild OA, but none of the three sham KKA^{Y} mice had signs of OA. The Figure shows one photomicroscopic image from each group illustrating the thin-section with the greatest signs of joint damage.
Conclusions: The KKA^{Y} mice (with control KKaa siblings) are a suitable mouse model of type 2 diabetes for the purpose of testing the effects of diabetes on OA without the presence of confounding factors such as a

610
IDENTIFICATION OF A GENE SIGNATURE FOR OSTEOARTHRITIS BY COMPARING MICROARRAY DATA FROM RODENT AND HUMAN CARTILAGE STUDIES

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Purpose: We were interested to compare the published gene sets of microarray studies on cartilage samples from rodent models of surgically induced and spontaneous osteoarthritis (OA) and to compare it to published gene sets from human osteoarthritic cartilage, in order to:
1) demonstrate the usefulness of animal models in osteoarthritis, and
2) to determine whether there are genes that are dysregulated in osteoarthritis regardless of species and models used.
Methods: We used gene sets that adhered to the following criteria:
1) Was from pure cartilage or from samples enriched for cartilage
2) The species of origin was mouse, rat or human
3) We preferred surgical models that induce knee instability, but a study for spontaneous osteoarthritis was also examined.
The study was gene centric, rather than probe centric and the genes mapping to the probes for each microarray was used for annotation purposes. For comparison of the different species, all genes were converted to the mouse ENTREZ gene ids. The common data sets were examined for gene ontology term enrichment and KEGG pathway enrichment.
Results: We identify eleven genes that are commonly up-regulated in data sets of mouse cartilage where osteoarthritis was surgically induced by destabilisation of the medial meniscus. Among the genes were Jag1 (A Notch ligand), Crispld2 and tetraspanin 2 (Tspan2). Interestingly, Crispld2 was the only gene in common between the surgically induced OA data sets and spontaneous OA in mice.
By comparing the KEGG pathways in common among the published data sets of surgically induced OA in rodents, focal adhesion, regulation of actin cytoskeleton and TGF beta signalling were the common ones in 3 out of the 4 microarrays compared.
In the four human studies we compared, changes in collagens genes were commonly present. Those were collagen IA1, IA2 , VA1 and XVA1. Furthermore, changes in Jag1, Aqp1, Thbs3, Ltbp2 and Tgfbi were among the genes present in three out of the four arrays examined.
There was not a single gene commonly dysregulated in all ten arrays examined. However, Ltbp2 was differentially regulated in eight out of the ten arrays studied. The next group included Collagen IA and IA2, fibronectin 1, Jag1, Timp3 and Tspan2 regulated in seven out of ten data sets.
Conclusions: We show that at least 313 genes are differentially regulated in 3 out of the 10 arrays compared, regardless of species, and model of osteoarthritis. From these, at least 29 genes were regulated in three out the four human microarrays examined.
Our analysis indicates an involvement of the TGF beta pathway in osteoarthritis and a central role of the actin cytoskeleton remodelling in the disease.
Due to the small number of overlap between different OA models it is difficult to determine a signature gene set that could discriminate between spontaneous and injury induced OA. However, our analysis indicates Ltbp2 and TSPAN2 as genes that merit further investigation as potential OA markers and possible involvement in OA pathogenesis.

Part of the gene signature identified		
Frequency	Gene symbol	Gene name
8	Ltbp2	Latent transforming growth factor beta binding protein 2
7	Col1a1	Collagen, type I, alpha 1
7	Col1a2	Collagen, type I, alpha 2
7	Col6a2	Collagen, type VI, alpha 2
7	Fn1	Fibronectin 1
7	Jag1	Jagged 1
7	Timp3	Tissue inhibitor of metalloproteinase 3
7	Tspan2	Tetraspanin 2
6	Emp1	Epithelial membrane protein 1
6	Inhba	Inhibin beta-A
6	MMP14	Matrix metallopeptidase 14 (membrane-inserted)
6	MMP2	Matrix metallopeptidase 2
6	Nbl1	Neuroblastoma, suppression of tumorigenicity 1
6	Pcolce	Procollagen C-endopeptidase enhancer protein

611
EVALUATION OF HUMAN SUBCHONDRAL BONE STRUCTURE USING VOLUMETRIC LOCAL BINARY PATTERNS METHOD

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Purpose: Osteoarthritis (OA) causes progressive degeneration of articular cartilage and pathological changes in subchondral bone. The bone adaptation due to decrease of cartilage thickness can be assessed volumetrically using micro-computed tomography (μCT) imaging. The local binary patterns (LBP) analysis is a promising method in the field of medical image processing as it is quite insensitive to monotonic greyscale variations. LBP allows performing the analysis of local structures both in 2D (planar) and 3D (volumetrically). Therefore, the aim of this study was to characterize the distribution of local patterns in the human subchondral bone at different stages of OA.

Methods: 18 osteochondral samples were prepared from 13 OA patients treated with total knee arthroplasty at Oulu University Hospital. Samples were stored in phosphate-buffered saline (PBS) and were scanned with μCT device at isotropic 27.8 μm voxel size (Skyscan 1172, Bruker microCT, Kontich, Belgium). Trabecular (figure 1A) and subchondral bone plates were manually segmented. After the μCT imaging, cylinders were formalin-fixed, parafin embedded and sectioned. Sections of 5 μm were stained with Safranin O. Histological sections were graded by three independent evaluators according to the standardized OARSI grading system and were averaged to obtain a final OARSI grade. Furthermore, LBP method was applied to the μCT scans to evaluate the distribution of local patterns. Briefly, the neighborhood of a center pixel is checked for evaluating the occurrences of equal/higher grey level values than in the center pixel. For each pixel, a specific local pattern is then determined based on the locations of these occurrences. The distribution of 2D local patterns was evaluated to estimate if some specific patterns appear or disappear at different stage of OA in the trabecular bone. Furthermore, the method was also applied volumetrically (26 neighbor pixels instead of 8) to evaluate a higher amount of different local patterns both in subchondral plate and trabecular bone.

Results: The range of OARSI grades of the samples was 1.33 - 6.25 (mean: 3.5 ± 1.8). The results of the analyzed parameters are presented in Table 1. The amount of different trabecular patterns assessed both in 2D and 3D showed that new patterns appear with the higher OA grades. The evaluation of specific trabecular 2D local patterns showed the apparition of complex patterns (more than 3 neighbor pixels with higher/equal value than studied pixel) with increasing OA grade. At the opposite, some simple patterns showed to disappear with higher OA grades. By using a condition of $|R|>0.65$ for relevant increase/decrease of specific patterns with increasing OA grade, 109 patterns oriented horizontally were recognized to occur more often, while 14 patterns oriented vertically were less common (figure 1B). The amount of different local patterns in subchondral plate was normalized to the volume of interest and assessed only volumetrically, showing a diminution of different local patterns for an increase in OA.

Conclusions: The present study presents for the first time the distribution of local patterns in the subchondral bone at different stages of OA using the LBP method. The results show the formation of "new" trabecular local patterns when the OARSI grade increases. During an increase of OA grade, the local trabecular patterns will become more complex and connect to each other horizontally, generating more different patterns connecting each others. In the subchondral plate, normalization was required as the volume of interest of the bone plate was very different from one sample to another, and it was highly related to the OARSI grade. Eventually, while the restricted amount of different local patterns that can be assessed in 2D (256 possibilities) was an issue for normalization, the volumetric analysis was able to solve this problem as it allows the recognition of substantially higher number of patterns (~67 millions possibilities). The amount of different local patterns in the subchondral plate decreased with inverse proportion to the OARSI grade. To conclude, this study shows the relevance of the LBP method in bone structure analysis related to OA. The obtained results reveal the adaptation of the trabecular bone to OA and suggest the possibility to assess OA level by evaluating the occurrences of specific local bone patterns.

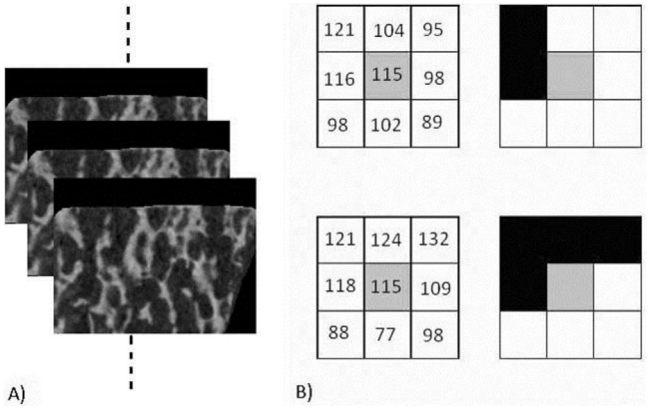

Figure 1. A) micro-CT scans of the segmented trabecular bone. B) Examples of studied pixels (in grey) and their corresponding local patterns. Up: typical patterns disappearing with OA; down: typical pattern appearing with OA.

Table 1
Correlation coefficients (Pearson) between LBP parameters and OARSI grades (N = 18)

Parameters	Correlation (highest)	Comments
Amount of different 3D patterns in trabecular bone	R=0.83	A threshold is applied to evaluate solely the bone and not the "empty spaces"
Amount of different 2D patterns in trabecular bone	R=0.87	A threshold is applied to evaluate solely the bone and not the "empty spaces"
2D Trabecular patterns appearing with OA	R>0.65 (R=0.87)	109 patterns recognized. Complexes and mostly oriented horizontally
2D Trabecular patterns disappearing with OA	R<-0.65 (R=−0.77)	14 patterns recognized. Simples and mostly oriented vertically
Amount of different 3D patterns in subchondral plate	R=−0.77	The amount of patterns is normalized by the volume of interest (related to thickness)
Amount of different 2D patterns in subchondral plate	Not significant	Result irrelevant after normalization due to the limited amount of possible patterns in 2D