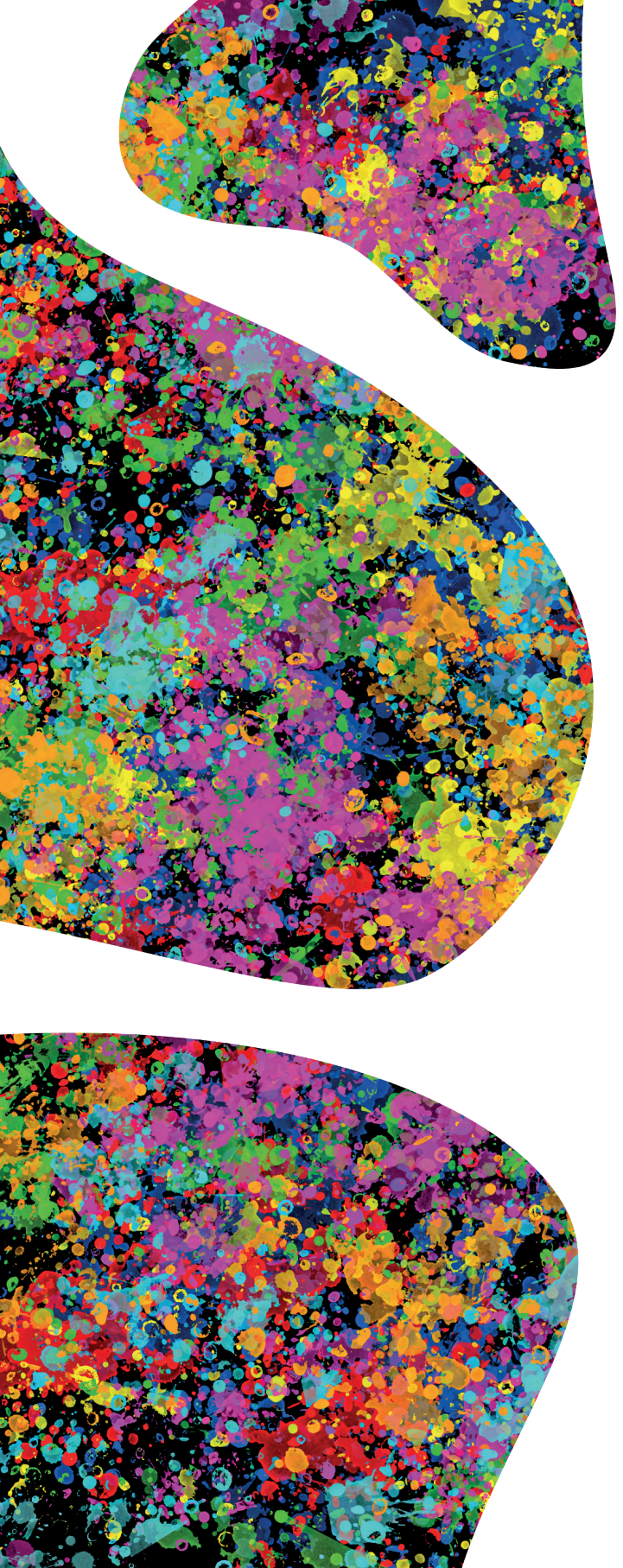
The bone and cartilage interplay in osteoarthritis: key to effective treatment strategy

Tuerlings, M.



CHAPTER 2

RNA sequencing reveals interacting key determinants of osteoarthritis acting in subchondral bone and articular cartilage

Margo Tuerlings[1], Marcella van Hoolwerff[1], Evelyn Houtman[1], H. Eka D. Suchiman[1], Nico Lakenberg[1], Hailiang Mei[1], Enrike H.M.J. van der Linden[2], Rob G.H.H. Nelissen[2], Yolande F.M. Ramos[1], Rodrigo Coutinho de Almeida[1], Ingrid Meulenbelt[1]

[1] Dept. of Biomedical Data Sciences, Leiden University Medical Center, Leiden, The Netherlands.
[2] Dept. Orthopaedics Leiden University Medical Center, Leiden, The Netherlands.

Arthritis & Rheumatology, volume 73, issue 5, May 2021, pages 789-799
DOI: 10.1002/art.41600

Abstract

Objective: To identify key determinants of the interactive pathophysiologic processes in subchondral bone and cartilage in osteoarthritis (OA).

Methods: We performed RNA sequencing on macroscopically preserved and lesioned OA subchondral bone from patients in the Research Arthritis and Articular Cartilage study who underwent joint replacement surgery due to OA (n = 24 sample pairs: 6 hips and 18 knees). Unsupervised hierarchical clustering and differential expression analyses were conducted. Results were combined with data on previously identified differentially expressed genes in cartilage (partly overlapping samples) as well as data on recently identified OA risk genes.

Results: We identified 1569 genes significantly differentially expressed between lesioned and preserved subchondral bone, including *CNTNAP2* (fold change (FC)=2.4, false discovery rate (FDR)=$3.36x10^{-5}$) and *STMN2* (FC=9.6, FDR=$3.36x10^{-3}$). Among these 1569 genes, 305 were also differentially expressed, and with same direction of effects, in cartilage, including the recently recognized OA susceptibility genes *IL11* and *CHADL*. Upon differential expression analysis with stratification for joint site, we identified 509 genes exclusively differentially expressed in subchondral bone of the knee, including *KLF11* and *WNT4*. These genes that were differentially expressed exclusively in the knee were enriched for involvement in epigenetic processes, characterized by, e.g., *HIST1H3J* and *HIST1H3H*.

Conclusion: Among the most consistently differentially expressed genes with OA pathophysiology in both bone and cartilage were *IL11* and *CHADL.* As these genes were recently also identified as robust OA risk genes they classify as attractive druggable targets acting on two OA disease relevant tissues.

Introduction

Osteoarthritis (OA) represents multiple subtypes of degenerative joint diseases, characterized by progressive and irreversible degeneration of the articular cartilage and structural changes in the subchondral bone. Globally, OA is a highly prevalent and disabling disease that results in high social and economic burdens to society [1]. Yet, there is no proven therapy to prevent OA or slow down its progression. Development of OA is dependent on multiple factors, with both environmental and genetic components [2, 3]. To discover genes and underlying disease pathways, genetic investigations, such as large genome wide association studies, have been performed, identifying compelling OA risk single-nucleotide polymorphisms (SNPs) [4-6]. Functional follow-up studies involve exploring the expression patterns in disease-relevant tissues, behavior with pathophysiology, and/or expression quantitative trait locus (eQTL) or cis-eQTL analysis. To date, major efforts have been made to characterize pathophysiological processes of OA in articular cartilage. However, only few studies have focused on OA pathophysiologic processes in the underlying bone [7, 8].

In recent decades, there has been accumulating evidence that subchondral bone contributes to both onset and progression of OA [9-12]. In healthy bone, there is a balanced process between bone resorption and bone deposition, as a consequence of dynamic adaptation to mechanical load. In OA this balance is disturbed, which results in changes in the architecture of the subchondral trabecular bone, increased thickness of the subchondral bone plate, formation of new bony structures, called osteophytes, at the joint margins, and development of subchondral bone cysts [2, 13, 14]. In addition, studies have shown an association between the bone mineral density and development of OA, which suggest that subchondral bone is involved in the early stages of OA [13, 15]. This was also suggested by studies regarding subchondral bone marrow lesions, showing these to be very early markers of OA [8, 16].

In contrast to cartilage and despite its relevance, only a limited number of studies have focused on the characterization of OA disease processes at the gene expression level in subchondral bone. Chou et al. [7] performed whole-genome expression profiling of non-OA and OA subchondral bone using microarray analysis, which led to identification of genes involved in pathways such as lipid metabolism and mineral metabolism. Kuttapitiya et al. [8] used microarray analysis to identify genes involved in bone remodeling, pain sensitization, and matrix turnover being differentially expressed between OA bone marrow lesioned tissue and controls. However, both of these studies included samples from the knee only.

In the present study, we explored RNA sequencing data on preserved and lesioned OA

subchondral bone to identify genes that change with progression of OA. The samples used were obtained from the joints of patients in the Research Arthritis and Articular Cartilage (RAAK) study who underwent total joint replacement surgery due to OA. In total, we compared paired subchondral bone samples (preserved and lesioned) from 24 OA patients from whom preserved and lesioned cartilage was also collected. The results presented here contribute to further understanding of the ongoing OA process in the subchondral bone and provide give insight into the pathophysiology of the disease in bone relative to cartilage.

Methods

Sample description

The current investigation includes 26 participants from the RAAK study who underwent joint replacement surgery due to OA. Macroscopically preserved and lesioned OA subchondral bone was collected from the joints of these patients. Of note, classification of OA subchondral bone as preserved or lesioned was based on classification of its overlying cartilage as preserved or lesioned, as described previously [17]. The results reported here were compared to the results of our earlier study of macroscopically preserved and lesioned OA articular cartilage from 35 patients from the RAAK study [18]. Fourteen of these 35 patients were included in the present study, as samples of both preserved and lesioned subchondral bone and preserved and lesioned articular cartilage was available. The sample size for the current study was determined using the R package ssize.fdr v1.2 [19], with parameters based on our previous similar analysis of articular cartilage [18] and a desired power of 0.8 (Supplementary Figure 1). Since the parameters were based on cartilage, whereas bone is known to be more heterogeneous, we decided to include an excess of samples. The samples were either randomly selected or selected based on their overlap with the cartilage data. Informed consent was obtained from all participants in the RAAK study, and ethical approval for the RAAK study was granted by the medical ethics committee of Leiden University Medical Center (P08.239/P19.013).

RNA sequencing

RNA was isolated from subchondral bone using an RNeasy Mini Kit (Qiagen). Paired-end 2×100 bp RNA-sequencing (Illumina TruSeq RNA Library Prep Kit, Illumina HiSeq2000 and Illumina HiSeq4000) was performed. Strand-specific RNA-seq libraries were generated, which yielded a mean of 20 million reads per sample. Data from both Illumina platforms were integrated and analyzed with the same in-house pipeline. RNA-seq reads were aligned using GSNAP [20] against GRCh38, with default parameters. Read abundances per sample was estimated using HTSeq count v0.11.1 [21]. Only uniquely mapping reads were used for estimating expression. The quality of the raw

reads for RNA-seq was checked using MultiQC v1.7. [22] The adaptors were clipped using Cutadapt v1.1 [23], applying default settings (min overlap 3, min length). To identify outliers, principal component analysis (PCA) and hierarchical clustering on the samples were applied, and one extreme outlier was identified. A sensitivity analysis was performed, which showed that the outlier had a large effect on the results in the overall data set. Based on this, the outlier was removed from the data set. There was one sample without paired data, which was also removed from the data set. After removal of these samples, only 24 participants were included for further analysis. The RNA-seq data are deposited at the European Genome-Phenome Archive (accession number: EGAS00001004476).

Cluster analysis

Prior to the cluster analysis, variance stabilizing transformation was performed on the data and 1000 genes were selected based on the highest coefficient of variation [24, 25]. To identify the optimal number of clusters in the unsupervised hierarchical clustering the silhouette width score approach was used, with a higher average silhouette width score indicating a more optimal number of clusters [26]. Details on the cluster analyses and the stability of cluster solutions have been reported previously [25].

Differential expression analysis and pathway enrichment

Differential expression analysis was performed on paired lesioned and preserved subchondral bone samples, using the DESeq2 R package, version 1.24.0 [27]. A general linear model assuming a negative binomial distribution was applied, followed by a paired Wald-test between lesioned and preserved OA samples, with the preserved samples set as a reference. The Benjamini-Hochberg method was used to correct for multiple testing, as indicated by the false discovery rate (FDR), with a significance cutoff value of 0.05. Gene enrichment was performed using the online functional annotation tool DAVID, selecting for the gene ontology terms Biological Processes (GOTERM_BP_DIRECT), Cellular Component (GOTERM_CC_DIRECT) and Molecular Function (GOTERM_MF_DIRECT) and for the Reactome Homo Sapiens (R-HAS) and the KEGG pathways [28]. Moreover, the protein-protein interactions were analyzed using the online tool STRING, version 11.0 [29]. An analysis summary scheme is shown in **Figure 1**.

RT-qPCR validation

Complementary DNA (cDNA) synthesis was performed using Transcriptor First Strand cDNA Synthesis Kit (Roche), using 400 ng of RNA. We used RT-qPCR to quantitatively determine gene expression of *FRZB, CNTNAP2, STMN2, CHRDL2, POSTN*, and *ASPN.* Relative gene expression was evaluated using -ΔCT values, using *GAPDH* and *SDHA* as internal controls. Generalized estimating equation (GEE) analysis was performed to

calculate the significance of differences between the lesioned and preserved samples.

Comparison subchondral bone and articular cartilage

The 1569 genes that were significantly differentially expressed (by FDR) between preserved and lesioned OA subchondral bone (24 paired samples) reported here were compared to the 2387 genes that were significantly differentially expressed between preserved and lesioned OA articular cartilage (35 paired samples) as determined in our earlier study [18]. Genes that were significantly differentially expressed in both tissues were selected, and the directions of effect were explored.

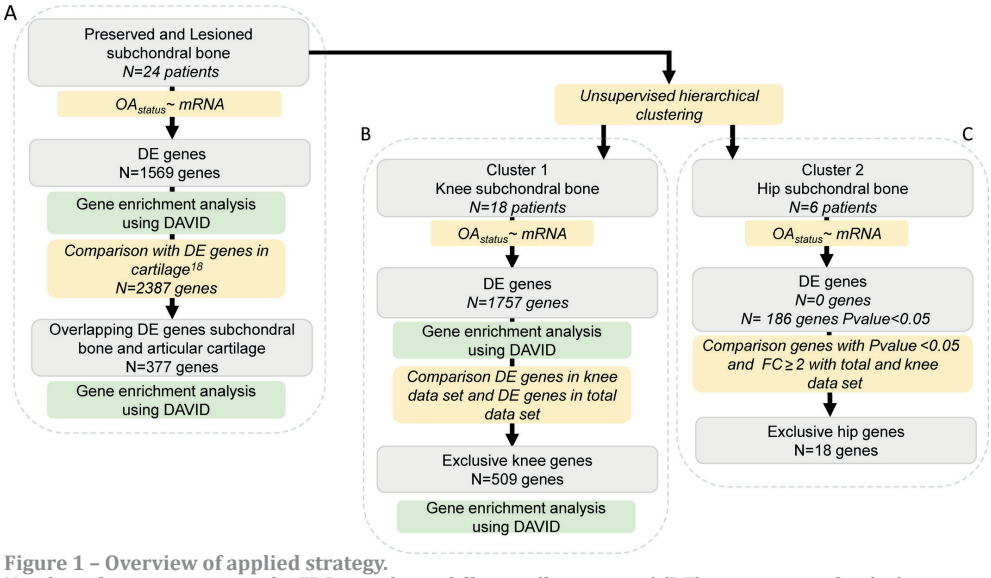


Figure 1 – Overview of applied strategy.
Number of genes represents the FDR-significant differentially expressed (DE) genes, except for the hip genes.

Results

Sample characteristics

To characterize the pathophysiologic process in subchondral bone with ongoing OA, we performed RNA-seq on macroscopically preserved and lesioned OA subchondral bone samples from patients in the RAAK study who underwent joint replacement surgery due to OA. The RNA-seq was performed on 24 paired samples (6 from hips and 18 from knees, **Supplementary Table 1**).

Prior to the differential expression analysis, we tested possible contamination of cartilage tissue in the subchondral bone samples. We used RNA-seq data on both tissue types from the same joint and evaluated the relative difference in expression levels of three cartilage-specific genes (*COL2A1, COMP, CRTAC1*) and three bone-specific genes (*COL1A1, SPP1, BGLAP*), as described previously [30]. As shown in **Supplementary Table 2**, we observed relatively low levels of cartilage-specific genes and high levels

of bone-specific genes in the subchondral bone data set under study, suggesting no-to-minimal cross-contamination. Next, we explored whether the expression pattern in subchondral bone was associated with any baseline characteristics of the patients (**Supplementary Table 1**), by performing unsupervised hierarchical clustering. To include the most informative genes in the cluster analysis, 1000 genes were selected based on the highest coefficient of variation in the total data set (preserved and lesioned, N=24 pairs). As shown in **Figure 2** and **Supplementary Figure 2**), we identified two clusters. These appeared to be based on joint site, indicating an inherent difference between hip and knee subchondral bone.

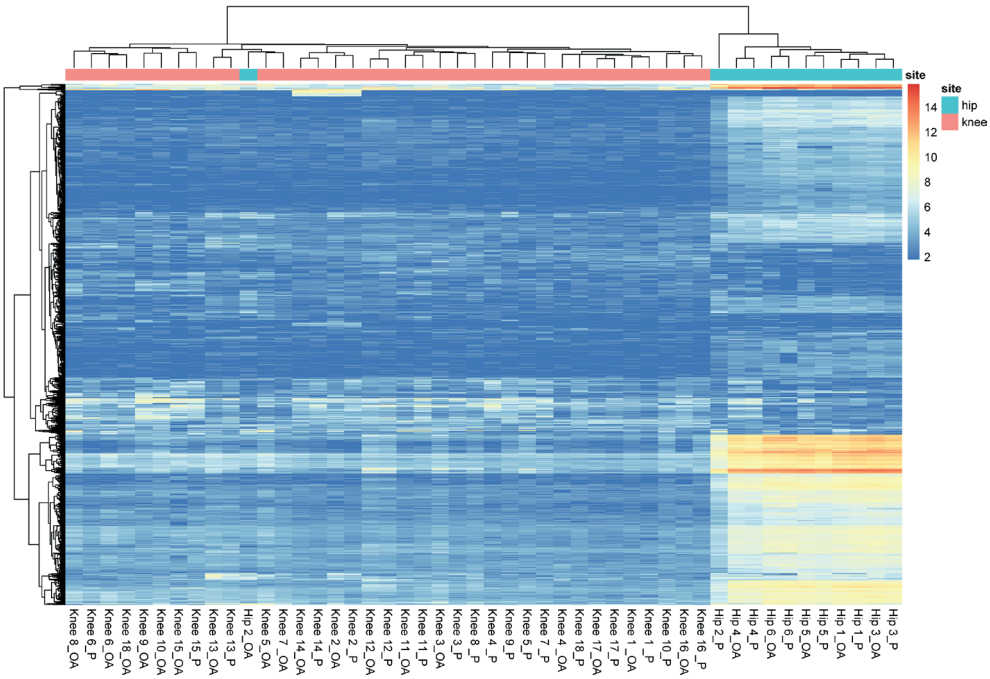


Figure 2 – Cluster analysis based on the 1000 genes selected for their highest COV.
Two clusters were identified based on knee samples (left) and hip samples (right).

Differential expression analysis and pathway enrichment

We first determined the genes that were consistently differentially expressed between preserved and lesioned OA subchondral bone in the overall data set, to explore the most consistent OA pathways (**Figure 1A**). Upon differential expression analysis in the 24 samples, we identified 1569 genes that were genome-wide significantly differentially expressed between lesioned and preserved OA subchondral bone tissue. Of these differentially expressed genes, 750 were up-regulated and 819 were down-regulated (**Figure 3** and **Supplementary Table 3**). The most significantly down-regulated gene was *FRZB* (FC=0.53, FDR=3.99x10^{-7}), encoding the frizzled-related protein, which is

a well-known OA gene showing consistently lower expression in lesioned relative to preserved OA articular cartilage [17, 18]. The most significantly up-regulated gene was *CNTNAP2* (FC=2.42, FDR=3.36x10^{-5}), encoding the contactin-associated protein-like 2 protein (CASPR2). Among the 1569 differentially expressed genes, 53 genes had an absolute FC of ≥2 (35 up-regulated and 18 down-regulated). The most highly up-regulated gene was *STMN2* (FC=9.56, FDR=2.36x10^{-3}), encoding stathmin 2, while the most down-regulated gene was *CHRDL2* (FC=0.14, FDR=1.20x10^{-4}), encoding chordin-like protein 2.

Next, we explored whether the 1569 significant differentially expressed genes were enriched in relation to particular pathways or processes, using DAVID. The results demonstrated significantly enriched Gene Ontology (GO) terms regarding processes involved in translational and posttranslational processes, such as signal recognition particle-dependent co-translational protein targeting to membrane (GO:0006614, 33 genes, FDR=4.27x10^{-7}) and translational initiation (GO:0006413, 36 genes, FDR=1.95x10^{-4}). These processes were both mainly characterized by ribosomal proteins such as *RPS24*, *RPS4X* and *RPS18* (**Supplementary Table 4)**. Gene enrichment analysis of the genes selected for the highest FC (FC≥2, N=53 genes), showed significant enrichment of processes regarding the extracellular matrix (GO:0005615, 16 genes, FDR=1.19x10^{-5}), characterized by the up-regulation of *WNT16* (FC=4.35, FDR= 6.88x10^{-4}) , *CRLF1* (FC=2.32, FDR=2.86x10^{-2}) and *OGN* (FC=3.43, FDR= 4.62x10^{-3}),

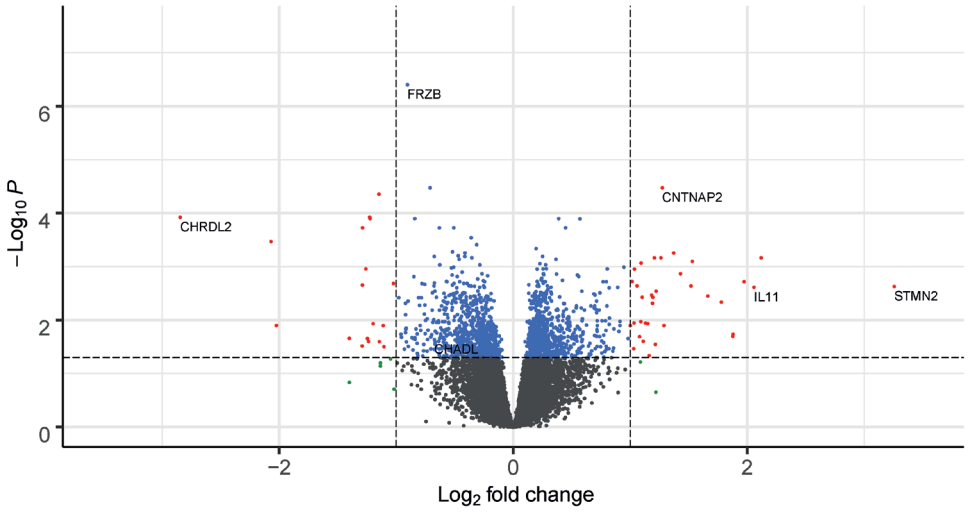


Figure 3 – Volcano plot of differential expressed genes in the subchondral bone.
The dots in the figure represent genes expressed in bone. Blue dots represent genes that are significantly differentially expressed, red dots represent genes that are significantly differentially expressed and have an absolute fold change of 2 or higher, and green dots represent the genes with an absolute fold change of two or higher that are not significantly differentially expressed.

and the proteinaceous extracellular matrix (GO:0005578, 7 genes, FDR=4.50x10^{-2}), characterized by up-regulation of *POSTN* (FC=2.04, FDR=3.44x10^{-2}), *ASPN* (FC=3.17, FDR=3.56x10^{-3}) and *CTHRC1* (FC=2.15, FDR=3.75x10^{-3}) (**Supplementary Table 5**). To explore interactions between proteins encoded by the 53 differentially expressed genes with an FC of ≥2, we used the online tool STRING. We identified significant enrichment for protein-protein interactions (PPI) among 22 of 44 proteins (P=3.20x10^{-9}, **Figure 4**).

Comparison subchondral bone and articular cartilage

To investigate interacting OA pathophysiologic processes in subchondral bone and articular cartilage, we compared differentially expressed genes identified in bone with our previously reported results on differentially expressed genes in articular cartilage [18] (**Figure 1A**, 24 sample pairs from bone and 35 from cartilage; 14 patients with available sample pairs from both bone and cartilage). This analysis revealed 337 genes that were differentially expressed in both subchondral bone and articular cartilage (**Supplementary Figure 3**). Of these 337 overlapping genes, the majority (305 genes) showed similar directions of effect in cartilage and bone (**Supplementary Table 6**), while 32 genes showed opposite directions of effect between the two tissue types

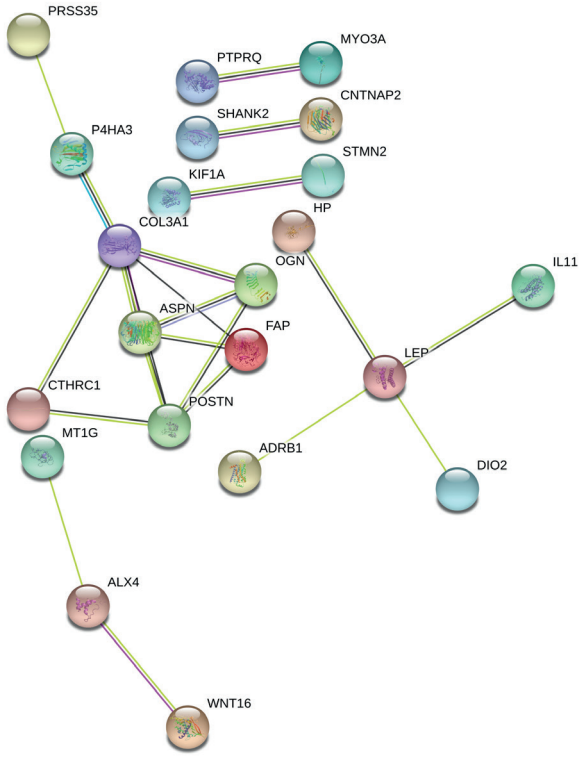


Figure 4 – Protein-protein interaction network of proteins encoded by genes that show an absolute fold change of 2 or higher (N=53 genes) created by STRING.

(**Supplementary Table 7**). *ALX4,* encoding aristaless-like homeobox 4, was notable gene among the genes showing opposite directions of effects. *ALX4* is known to be involved in osteogenesis and was one of the most highly up-regulated genes in bone (**Table 1**). Among the 305 genes showing similar direction of effects, 14 were among the top 25 genes with the highest FC in both tissues, such as *WNT16*, *IL11*, *CRLF1* and *FRZB* (**Table 1**).

To explore common underlying pathways in subchondral bone and articular cartilage, we performed gene enrichment analysis with the 305 genes that showed similar directions of effect in cartilage and bone. We found significant enrichment for the GO terms extracellular region (GO:0005576, 36 genes, FDR= $4.56x10^{-3}$), characterized by the expression of, for example, *COL6A3*, *FGF14* and *GDF6*, proteinaceous extracellular matrix (GO:0005578, 17 genes, FDR= $7.98x10^{-3}$), characterized by the expression of, for example, *CHADL*, *ADAMTS17* and *SPOCK3,* and extracellular space (GO:0005615, 37 genes, FDR= $4.42x10^{-3}$), characterized by the expression of, for example, *CD63*, *SPP1* and *RELN* (**Supplementary Table 8**).

Differential expression analysis stratified for joint site

Since hip and knee samples showed different gene expression profiles in the cluster analysis (**Figure 2**), we repeated the differential expression analysis with stratification by joint site to explore whether we could identify exclusive OA pathways that occur in subchondral bone of knees only or hips only. Differential expression analysis of the 18 knee sample pairs revealed 1757 genes that were significantly differentially expressed (**Figure 1B**), of which 902 genes were up-regulated and 855 genes were down-regulated in lesioned compared to preserved OA subchondral bone (**Supplementary Table 9**). Moreover, we identified 509 genes that were differentially expressed exclusively in the knee (**Supplementary Table 10**); i.e. these genes were not differentially expressed in analysis of the total data set (**Supplementary Table 3**) or the hip data set (**Supplementary Table 12**). Enrichment analysis of these genes that were differentially expressed exclusively in the knee showed significant enrichment for processes involved in epigenetic regulation, such as nucleosome (GO:0000786, 20 genes, $1.81x10^{-9}$), DNA methylation (R-HSA-5334118, 15 genes, $2.48x10^{-6}$) and regulation of gene silencing (GO:0060968, 6 genes, $1.90x10^{-2}$), all characterized by members of H3 histone family, such as *HIST1H3J* and *HIST1H3H* (**Supplementary Table 11).**

Differential expression analysis using only the hip samples (6 pairs) did not reveal any genes that were significantly differentially expressed by the FDR method when comparing preserved and lesioned subchondral bone (**Figure 1C**). However, among the genes with a P-value <0.05 and an absolute FC ≥2 (**Supplementary Table 12),** 18

Table 1 – Genes that belonged to the top 25 genes based on the highest absolute foldchange in either bone or cartilage.
Of these genes, 14 appear to be in the top 25 highest FC genes in both tissues.

		Subchondral bone		Articular Cartilage		Top 25 absolute foldchange	
Ensemble ID	**Gene name**	**FC**	**FDR**	**FC**	**FDR**	**SB**	**AC**
ENSG00000002745	WNT16	4,35	$6,88x10^{-4}$	8,48	$1,10x10^{-13}$	x	x
ENSG00000095752	IL11	4,16	$2,44x10^{-3}$	22,8	$1,53X10^{-20}$	x	x
ENSG00000156466	GDF6	3,67	$2,02x10^{-2}$	1,58	$3,19x10^{-2}$	x	
ENSG00000106809	OGN	3,43	$4,62x10^{-3}$	2,00	$1,02x10^{-3}$	x	
ENSG00000106819	ASPN	3,17	$3,56x10^{-3}$	1,65	$3,04x10^{-2}$	x	
ENSG00000095777	MYO3A	2,44	$1,27x10^{-2}$	2,25	$1,16x10^{-4}$	x	
ENSG00000006016	CRLF1	2,32	$2,86x10^{-2}$	3,04	$2,96x10^{-10}$	x	x
ENSG00000151025	GPR158	2,31	$6,88x10^{-4}$	2,73	$3,63x10^{-3}$	x	x
ENSG00000198729	PPP1R14C	2,19	$1,14x10^{-2}$	2,52	$1,33x10^{-11}$	x	
ENSG00000125144	MT1G	2,16	$2,50x10^{-2}$	1,97	$1,72x10^{-4}$	x	
ENSG00000052850	ALX4	2,08	$2,30x10^{-3}$	0,55	$2,75x10^{-2}$	x	
ENSG00000149380	P4HA3	2,05	$1,12x10^{-3}$	1,84	$1,49x10^{-5}$	x	
ENSG00000078098	FAP	2,05	$1,14x10^{-2}$	1,69	$1,09x10^{-3}$	x	
ENSG00000133110	POSTN	2,04	$3,44x10^{-2}$	2,06	$3,20x10^{-2}$	x	
ENSG00000230148	HOXB-AS1	2,00	$1,27x10^{-2}$	1,64	$4,86x10^{-2}$	x	
ENSG00000112984	KIF20A	1,97	$2,22x10^{-2}$	1,59	$4,44x10^{-2}$	x	
ENSG00000123610	TNFAIP6	1,93	$1,03x10^{-3}$	3,58	$2,48x10^{-8}$	x	x
ENSG00000178752	ERFE	1,87	$1,63x10^{-2}$	3,44	$8,82x10^{-12}$	x	x
ENSG00000148344	PTGES	1,64	$1,63x10^{-2}$	3,06	$3,61x10^{-12}$		x
ENSG00000006327	TNFRSF12A	1,50	$2,31x10^{-2}$	2,68	$1,14x10^{-8}$		x
ENSG00000169884	WNT10B	1,49	$3,25x10^{-2}$	3,47	$1,52x10^{-6}$		x
ENSG00000100473	COCH	1,46	$4,21x10^{-2}$	3,30	$1,01x10^{-8}$		x
ENSG00000196352	CD55	1,46	$2,48x10^{-2}$	2,96	$1,05x10^{-14}$		x
ENSG00000090530	P3H2	1,37	$1,14x10^{-2}$	3,23	$4,71x10^{-18}$		x
ENSG00000134259	NGF	1,36	$3,26x10^{-2}$	4,91	$2,53x10^{-14}$		x
ENSG00000118785	SPP1	1,36	$4,81x10^{-2}$	3,14	$8,98x10^{-7}$		x
ENSG00000140538	NTRK3	0,70	$3,56x10^{-3}$	0,31	$2,64x10^{-5}$		x
ENSG00000048540	LMO3	0,58	$3,82x10^{-3}$	0,28	$1,67x10^{-5}$		x
ENSG00000162998	FRZB	0,53	$3,99x10^{-7}$	0,27	$1,87x10^{-9}$	x	x
ENSG00000189056	RELN	0,53	$2,56x10^{-2}$	0,22	$7,37x10^{-12}$	x	x
ENSG00000141469	SLC14A1	0,53	$1,71x10^{-2}$	0,51	$7,05x10^{-6}$	x	
ENSG00000121005	CRISPLD1	0,51	$1,84x10^{-2}$	0,36	$9,29x10^{-6}$	x	x
ENSG00000187595	ZNF385C	0,51	$3,82x10^{-3}$	0,43	$2,30x10^{-6}$	x	
ENSG00000124440	HIF3A	0,49	$2,07x10^{-3}$	0,58	$2,72x10^{-2}$	x	
ENSG00000259916	AL845331.2	0,46	$3,16x10^{-2}$	0,34	$3,50x10^{-2}$	x	x
ENSG00000179399	GPC5	0,43	$1,27x10^{-4}$	0,36	$1,47x10^{-8}$	x	x
ENSG00000223561	AC005165.1	0,43	$1,20x10^{-4}$	0,45	$5,31x10^{-4}$	x	
ENSG00000102466	FGF14	0,41	$1,89x10^{-4}$	0,58	$2,01x10^{-4}$	x	
ENSG00000256995	AC084816.1	0,38	$2,20x10^{-2}$	0,45	$2,20x10^{-5}$	x	

		Subchondral bone		Articular Cartilage		Top 25 absolute foldchange	
Ensemble ID	**Gene name**	**FC**	**FDR**	**FC**	**FDR**	**SB**	**AC**
ENSG00000130294	KIF1A	0,25	$1,27x10^{-2}$	0,37	$8,64x10^{-8}$	x	x
ENSG00000196104	SPOCK3	0,24	$3,41x10^{-4}$	0,22	$1,56x10^{-9}$	x	x
ENSG00000054938	CHRDL2	0,14	$1,20x10^{-4}$	0,13	$7,07x10^{-9}$	x	x

genes appeared to be differentially expressed exclusively in the hip; i.e. not differentially expressed in an analysis of the total data set (**Supplementary Table 3**) or the knee dataset (**Supplementary Table 9**). Included among these genes with differential expression exclusively in the hip were *CALCR*, *LGR5* and *COL2A1* (**Supplementary Table 13**).

Validation of differentially expressed genes

To validate and replicate the findings of the differential expression analysis performed using RNA-seq, we used a set of 20 samples to conduct both technical replication (10 samples) and biological replication (10 samples) by RT-qPCR. Validation analysis of six genes, *FRZB*, *CNTNAP2*, *STMN2*, *CHRDL2*, *POSTN*, and *ASPN,* showed significant differences between preserved and lesioned subchondral bone, with directions of effects similar to those found by RNA-seq. Replication analysis also showed significant differences, with the same direction of effects as shown by RNA-seq (**Supplementary Table 14**).

Differential expression of previously identified risk genes

In recent genome-wide association studies of hip and knee OA [5, 6], 27 loci conferring risk to OA were identified (**Table 2**). To assess whether those OA susceptibility genes are also involved in OA pathophysiology in articular cartilage, subchondral bone, or both, we explored their expression levels and differential expression between lesioned and preserved tissue in our data sets. As shown in **Table 2**, we identified two risk genes, *IL11* and *CHADL,* that were differentially expressed in both subchondral bone and articular cartilage. In addition, *IL11* showed both significant differential expression in knee subchondral bone (FC=4.07, FDR=$7.00x10^{-3}$) and a high FC (FC=4.77, Pval= $4,43x10^{-02}$) in hip subchondral bone. This indicates that, based on our data sets, *IL11* has an effect in both tissues and at both joint sites, albeit not significant according to FDR in hip subchondral bone.

Discussion

Differential expression analysis of gene expression levels in preserved and lesioned OA subchondral bone (N=24 paired samples) revealed 1569 genes that were significantly

Table 2 – Expression levels and differential expression of new risk genes reported in two recent GWAS.

	Bone - total dataset			Cartilage - total dataset		
	Expression*	FC P vs. OA	FDR	Expression*	FC P vs. OA	FDR
COL11a1	1	1,19	$6{,}21x10^{-1}$	1	1,07	$7{,}59x10^{-1}$
HDAC9	2	0,97	$6{,}75x10^{-1}$	1	0,59	$9{,}10x10^{-6}$
SMO	2	1,00	$9{,}91x10^{-1}$	1	0,69	$7{,}85x10^{-5}$
TNC	1	1,18	$2{,}58x10^{-1}$	1	1,41	$1{,}09x10^{-2}$
LMX1B	Not expressed	NA	NA	3	0,99	$9{,}80x10^{-1}$
LTBP3	1	0,87	$3{,}08x10^{-1}$	1	1,08	$6{,}95x10^{-1}$
FAM101A (RFLNA)	4	0,99	$9{,}77x10^{-1}$	2	0,49	$6{,}48x10^{-5}$
IL11	3	4,16	$2{,}44x10^{-3}$	1	22,80	$1{,}53x10^{-20}$
ITIH1	Not expressed	NA	NA	Not expressed	NA	NA
FILIP1	2	0,84	$7{,}07x10^{-2}$	3	1,23	$2{,}38x10^{-1}$
RUNX2	1	1,07	$4{,}75x10^{-1}$	2	0,93	$7{,}79x10^{-1}$
ASTN2	4	0,87	$2{,}42x10^{-1}$	4	0,82	$2{,}43x10^{-1}$
SMAD3	1	0,93	$3{,}74x10^{-1}$	1	0,84	$2{,}83x10^{-2}$
HFE	3	1,01	$9{,}07x10^{-1}$	2	0,88	$1{,}32x10^{-1}$
CHADL	4	0,63	$2{,}33x10^{-2}$	1	0,63	$1{,}29x10^{-2}$
LTBP1	1	0,97	$6{,}91x10^{-1}$	1	1,15	$1{,}70x10^{-1}$
SBNO1	1	0,98	$6{,}79x10^{-1}$	1	1,10	$3{,}94x10^{-1}$
WWP2	1	0,82	$2{,}47x10^{-1}$	1	0,79	$3{,}43x10^{-2}$
GDF5	4	0,92	$8{,}08x10^{-1}$	1	1,23	$3{,}09x10^{-1}$
TGFB1	Not expressed	NA	NA	Not expressed	NA	NA
TNFSF15	4	1,23	$2{,}42x10^{-1}$	3	1,00	$9{,}91x10^{-1}$
FGF18	Not expressed	NA	NA	2	1,58	$9{,}51x10^{-4}$
CTSK	1	1,41	$3{,}23x10^{-1}$	1	1,03	$8{,}91x10^{-1}$
DPEP1 (MBD1)	1	0,95	$2{,}83x10^{-1}$	1	0,96	$6{,}20x10^{-1}$
DIABLO	4	0,95	$7{,}37x10^{-1}$	3	1,08	$6{,}46x10^{-1}$
CRHR1	Not expressed	NA	NA	4	0,62	$5{,}10x10^{-1}$
MAPT	3	0,61	$3{,}47x10^{-2}$	4	0,70	$1{,}56x10^{-1}$

* Expression in quartiles, with 1 being the highest expressed quartile and 4 being the lowest expressed quartile

differentially expressed, including *CNTNAP2* and *STMN2*. Upon comparing these 1569 differentially expressed genes with the 2387 genes previously shown to be differentially expressed with OA pathophysiology in cartilage, we found an overlap of 305 genes that had the same direction of effect. These 305 overlapping genes were enriched for processes related to the extracellular matrix, characterized by the expression of, amongst others, *COL6A3, GDF6* and *SPP1*. Moreover, among the 305 overlapping genes were *IL11* and *CHADL* (**Supplementary Table 6**), which were previously identified as

being OA risk genes (**Table 2**). By applying hierarchical clustering on the overall RNA-seq data set from subchondral bone, we observed two clusters based on joint site (knee and hip). When stratifying the analysis for joint site, we identified 1759 genes that were differentially expressed between preserved and lesioned knee OA bone, 509 of which were differentially expressed in the knee exclusively, including genes such as *WNT4* and *KLF11*. These OA genes that were differentially expressed exclusively in the knee were enriched for regulation of gene silencing by epigenetic processes, such as DNA methylation and histone modification, characterized by genes such as *HIST1H3J* and *HIST1H3H*, as well as being enriched for other processes.

Among the 1569 genes that were significantly differentially expressed between lesioned and preserved OA subchondral bone using the FDR method in the complete data set, we identified *CNTNAP2* (FC=2.42, FDR=$3.36x10^{-5}$) and *STMN2* (FC=9.56, FDR=$2.36x10^{-3}$) as the most significantly up-regulated gene and the gene with the highest FC, respectively. *CNTNAP2*, encoding CASPR2, is known for its effect on cell-cell interactions in the nervous system, synapse development, neural migration, and neural connectivity [31, 32]. Neither *CNTNAP2* nor its encoded protein were previously identified as being related to OA. *STMN2* also plays a role in the control of neuronal differentiation. Moreover, *STMN2* is expressed during osteogenesis and it was previously shown to be highly up-regulated in OA bone marrow lesions as compared to control bone samples [8, 33]. In addition, we found other neural markers to be up-regulated in lesioned compared to preserved OA subchondral bone, such as *NGF* and *THBS3* (**Supplementary Table 3**). Based on these findings, we hypothesize that the formation of new neuronal structures in bone is increased with ongoing OA, which might suggest that OA-related pain originates from bone [8]. However, functional follow up research is needed to confirm this hypothesis.

The hierarchical clustering was done on the top 1000 genes that showed the highest coefficient of variation between samples; hence, the clusters reflect particularly large differences. Based on the results observed here, it could thus be concluded that these highly variable genes reflect consistent differences between subchondral bone in the knees and subchondral bone in the hip, which was not previously seen in similar analyses of the cartilage [25]. Consequently, the fact that neither preserved and lesioned samples from the same individual nor preserved samples or lesioned samples as a group cluster together, indicated that the 1000 genes with the highest coefficient of variation are marking differences between knees and hips only. This does not rule out the relevance of the highly consistently differentially expressed genes reflecting OA subchondral bone pathology described here.

Upon differential expression analysis with stratification by joint site, we discovered 509

genes that were unique to the knee compared to the complete data set, which were significantly enriched for epigenetic processes such as DNA methylation, reflected by the expression of, among others, *HIST1H3J* and *HIST1H3H*. The significant enrichment of these epigenetic processes among the knee-exclusive genes indicates a change in epigenetics with ongoing knee OA, which is not seen with ongoing hip OA. This was also previously demonstrated in articular cartilage, where hip and knee methylation profiles clustered apart irrespective of the OA status. However, this was characterized by the expression of different genes, such as the homeobox genes [34, 35]. We did not find FDR-significant genes when selecting the hip samples, which is likely due to the small sample size (6 sample pairs). Nonetheless, we identified 18 genes that were exclusively differentially expressed in the hip based on the nominal P-value and an absolute FC ≥2, including genes such as *CALCR*, *LGR5* and *COL2A1*. However, replication is needed to confirm our findings regarding these genes differentially expressed exclusively in the hip.

Given the accumulating awareness of cross-talk between articular cartilage and subchondral bone during OA [10, 36], we compared RNA-seq data from subchondral bone and from articular cartilage (24 sample pairs, and 35 sample pairs, respectively, with an overlap of 14 patients). Compared to the number of genes identified as being significantly differentially expressed between preserved and lesioned OA articular cartilage based on FDR (2387 genes), we found fewer genes that were significantly differentially expressed by FDR between preserved and lesioned OA subchondral bone (1569 genes). This difference might be due to the difference in sample size. However, it could also reflect the fact that bone as multicellular tissue is more heterogeneous. The relatively small overlap in genes that were differentially expressed in the same direction in both subchondral bone and cartilage (9.31%, 305 genes), suggest that there is a difference in OA pathophysiology between the two tissues.

To find genes that are most likely causal in OA, we explored 27 previously published genes with SNPs that were identified as being genome-wide significantly associated with OA (**Table 2**), suggesting that those genes have a more causal relationship to OA and making them attractive potential drug targets [5, 6]. To examine whether the previously identified OA risk genes are involved in the OA pathophysiological process in both tissues, we compared the expression levels and the differential expression between preserved and lesioned samples (**Table 2**). We found the OA risk genes *IL11* and *CHADL* were differentially expressed in both articular cartilage and subchondral bone and with the same direction of effect, thus making them attractive potential drug targets with effects in both tissues. *CHADL*, encoding chondroadherin-like protein, is involved in collagen binding and is a negative modulator of chondrocyte differentiation.

The OA susceptibility allele rs117018441-T, located in an intron of *CHADL*, marks higher expression of *CHADL* compared to rs117018441-G in skeletal muscle and adipose tissue according to the Genotype-Tissue Expression Project [5, 37]. This may indicate that increased expression of *CHADL* has a negative regulatory role both in bone and cartilage and that inhibition of this gene could be a therapeutic strategy. However, when stratifying for joint site, we found *CHADL* to be differentially expressed specifically in the knee subchondral bone, suggesting that it is a treatment target for knee OA exclusively.

IL11, encoding Interleukin 11 (IL-11), is known for its role in bone remodelling and lack of IL-11 function is associated with impaired bone formation [38]. Notably, IL-11 is recently proposed as potential therapeutic target for OA in cartilage [6], since OA risk allele rs4252548-T, a missense variant p.Arg112His, acts via reduced function of the IL-11 protein. As such, increasing IL-11 protein levels was proposed as a strategy for treatment of OA. In this study we have again shown that *IL11* is highly up-regulated in lesioned versus preserved OA tissue in both subchondral bone and articular cartilage (FC=4.16 and FC=22.8, respectively). Taken together, these data indicate that reduced function of IL-11 predisposes to OA onset and that the up-regulation of *IL11* with OA pathophysiology could be considered an attempt of the chondrocytes to enhance extracellular matrix integrity. Nonetheless, the consistent and considerable up-regulation of *IL11* in both subchondral bone and articular cartilage may not necessarily reflect a lack of potency to produce IL-11, unless translation of the protein is hampered. This requires further functional investigation preferably in an *in vitro* model of OA. *CHADL* and *IL11* could both be highly suitable treatment targets with effects in both bone and cartilage. However, further functional research is needed to confirm the effects of these genes on bone and cartilage metabolism.

The classification of OA subchondral bone as preserved or lesioned is derived from its overlying cartilage. We acknowledge that this ascertainment strategy is bound to introduce heterogeneity between samples. Nonetheless, we find FDR-significant, and hence very consistent, differentially expressed genes. In other words, despite the fact that there may be heterogeneity in the preserved cartilage, we found consistent markers of the OA pathophysiological process in subchondral bone.

To our knowledge, we are the first reported study of large-scale differential gene expression patterns in OA subchondral, performed using RNA-seq in both hip and knee samples. We identified distinct differences in expression patterns between hips and knees. Moreover, we identified multiple genes that were previously demonstrated in OA articular cartilage, in addition to genes that were subchondral bone specific. These results will contribute to a better understanding of the pathophysiological processes

underlying the development of OA.

Declarations

Acknowledgements

We thank all the participants of the RAAK study. The LUMC has and is supporting the RAAK study. We also thank Demien Broekhuis, Robert van der Wal, Peter van Schie, Shaho Hasan, Maartje Meijer, Daisy Latijnhouwers and Geert Spierenburg for collecting the RAAK material. We thank the personnel of the Sequence Analysis Support Core of Leiden University Medical Center for their support. Data were generated within the scope of the Medical Delta Regenerative Medicine 4D programs Generating Complex Tissues with Stem Cells and Printing Technology and Improving Mobility with Technology.

Funding

The study was funded by the Dutch Scientific Research Council (NOW/ZonMW Vici grant 91816631/528), the European Union Seventh Framework Programme (project TREAT-OA; 200800), and the Dutch Arthritis Society (DAA_10_1-402).

Disclosures

The authors have declared no conflicts of interest.

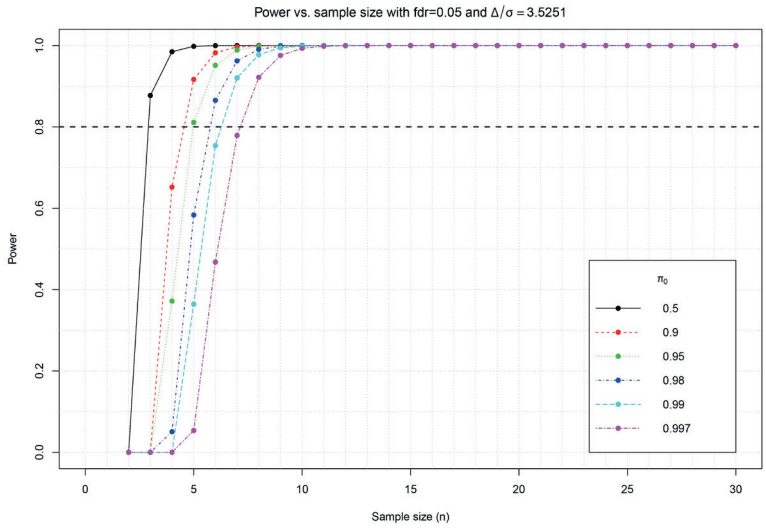
References

1. Woolf, A.D., J. Erwin, and L. March, *The need to address the burden of musculoskeletal conditions.* Best Pract Res Clin Rheumatol, 2012. **26**(2): p. 183-224.
2. Goldring, M.B. and S.R. Goldring, *Articular cartilage and subchondral bone in the pathogenesis of osteoarthritis.* Ann N Y Acad Sci, 2010. **1192**: p. 230-7.
3. Vina, E.R. and C.K. Kwoh, *Epidemiology of osteoarthritis: literature update.* Curr Opin Rheumatol, 2018. **30**(2): p. 160-167.
4. Zeggini, E., et al., *Identification of new susceptibility loci for osteoarthritis (arcOGEN): a genome-wide association study.* Lancet, 2012. **380**(9844): p. 815-23.
5. Styrkarsdottir, U., et al., *Meta-analysis of Icelandic and UK data sets identifies missense variants in SMO, IL11, COL11A1 and 13 more new loci associated with osteoarthritis.* Nat Genet, 2018. **50**(12): p. 1681-1687.
6. Tachmazidou, I., et al., *Identification of new therapeutic targets for osteoarthritis through genome-wide analyses of UK Biobank data.* Nat Genet, 2019. **51**(2): p. 230-236.
7. Chou, C.H., et al., *Genome-wide expression profiles of subchondral bone in osteoarthritis.* Arthritis Res Ther, 2013. **15**(6): p. R190.
8. Kuttapitiya, A., et al., *Microarray analysis of bone marrow lesions in osteoarthritis demonstrates upregulation of genes implicated in osteochondral turnover, neurogenesis and inflammation.* Ann Rheum Dis, 2017. **76**(10): p. 1764-1773.
9. Fellows, C.R., C. Matta, and A. Mobasheri, *Applying Proteomics to Study Crosstalk at the Cartilage-Subchondral Bone Interface in Osteoarthritis: Current Status and Future Directions.* EBioMedicine, 2016. **11**: p. 2-4.
10. Goldring, S.R. and M.B. Goldring, *Changes in the osteochondral unit during osteoarthritis: structure, function and cartilage-bone crosstalk.* Nat Rev Rheumatol, 2016. **12**(11): p. 632-644.
11. Funck-Brentano, T. and M. Cohen-Solal, *Crosstalk between cartilage and bone: when bone cytokines matter.* Cytokine Growth Factor Rev, 2011. **22**(2): p. 91-7.
12. Pan, J., et al., *Elevated cross-talk between subchondral bone and cartilage in osteoarthritic joints.* Bone, 2012. **51**(2): p. 212-7.
13. Funck-Brentano, T. and M. Cohen-Solal, *Subchondral bone and osteoarthritis.* Curr Opin Rheumatol, 2015. **27**(4): p. 420-6.
14. Loeser, R.F., et al., *Osteoarthritis: a disease of the joint as an organ.* Arthritis Rheum, 2012. **64**(6): p. 1697-707.
15. Barbour, K.E., et al., *Bone Mineral Density and the Risk of Hip and Knee Osteoarthritis: The Johnston County Osteoarthritis Project.* Arthritis Care Res (Hoboken), 2017. **69**(12): p. 1863-1870.
16. Roemer, F.W., et al., *Change in MRI-detected subchondral bone marrow lesions is associated with cartilage loss: the MOST Study. A longitudinal multicentre study of knee osteoarthritis.* Ann Rheum Dis, 2009. **68**(9): p. 1461-5.
17. Ramos, Y.F., et al., *Genes involved in the osteoarthritis process identified through genome wide expression analysis in articular cartilage; the RAAK study.* PLoS One, 2014. **9**(7): p. e103056.
18. Coutinho de Almeida, R., et al., *RNA sequencing data integration reveals an miRNA interactome of osteoarthritis cartilage.* Ann Rheum Dis, 2019. **78**(2): p. 270-277.

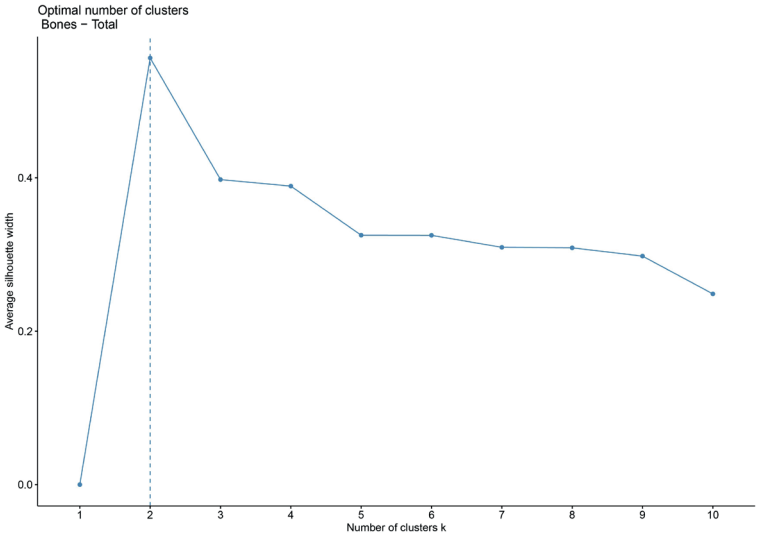
19. Liu, P. and J.T.G. Hwang, *Quick calculation for sample size while controlling false discovery rate with application to microarray analysis.* Bioinformatics, 2007. **23**(6): p. 739-746.
20. Wu, T.D. and C.K. Watanabe, *GMAP: a genomic mapping and alignment program for mRNA and EST sequences.* Bioinformatics, 2005. **21**(9): p. 1859-75.
21. Anders, S., P.T. Pyl, and W. Huber, *HTSeq--a Python framework to work with high-throughput sequencing data.* Bioinformatics, 2015. **31**(2): p. 166-9.
22. Ewels, P., et al., *MultiQC: summarize analysis results for multiple tools and samples in a single report.* Bioinformatics, 2016. **32**(19): p. 3047-8.
23. Martin, M., *Cutadapt removes adapter sequences from high-throughput sequencing reads.* 2011, 2011. **17**(1): p. 3 %J EMBnet.journal.
24. Li, W., M. Fan, and M. Xiong, *SamCluster: an integrated scheme for automatic discovery of sample classes using gene expression profile.* Bioinformatics, 2003. **19**(7): p. 811-817.
25. Coutinho de Almeida, R., et al., *Identification and characterization of two consistent osteoarthritis subtypes by transcriptome and clinical data integration.* Rheumatology (Oxford), 2020.
26. Charrad, M., et al., *NbClust: An R Package for Determining the Relevant Number of Clusters in a Data Set.* 2014, 2014. **61**(6): p. 36 %J Journal of Statistical Software.
27. Love, M.I., W. Huber, and S. Anders, *Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2.* Genome Biology, 2014. **15**(12): p. 550.
28. Huang da, W., B.T. Sherman, and R.A. Lempicki, *Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources.* Nat Protoc, 2009. **4**(1): p. 44-57.
29. Szklarczyk, D., et al., *STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets.* Nucleic Acids Res, 2019. **47**(D1): p. D607-d613.
30. Chou, C.H., et al., *Direct assessment of articular cartilage and underlying subchondral bone reveals a progressive gene expression change in human osteoarthritic knees.* Osteoarthritis Cartilage, 2013. **21**(3): p. 450-61.
31. Lu, Z., et al., *Molecular Architecture of Contactin-associated Protein-like 2 (CNTNAP2) and Its Interaction with Contactin 2 (CNTN2).* J Biol Chem, 2016. **291**(46): p. 24133-24147.
32. Rodenas-Cuadrado, P., J. Ho, and S.C. Vernes, *Shining a light on CNTNAP2: complex functions to complex disorders.* Eur J Hum Genet, 2014. **22**(2): p. 171-8.
33. Chiellini, C., et al., *Stathmin-like 2, a developmentally-associated neuronal marker, is expressed and modulated during osteogenesis of human mesenchymal stem cells.* Biochem Biophys Res Commun, 2008. **374**(1): p. 64-8.
34. Reynard, L.N., *Analysis of genetics and DNA methylation in osteoarthritis: What have we learnt about the disease?* Semin Cell Dev Biol, 2017. **62**: p. 57-66.
35. den Hollander, W., et al., *Knee and hip articular cartilage have distinct epigenomic landscapes: implications for future cartilage regeneration approaches.* Ann Rheum Dis, 2014. **73**(12): p. 2208-12.
36. Kwan Tat, S., et al., *Targeting subchondral bone for treating osteoarthritis: what is the evidence?* Best Pract Res Clin Rheumatol, 2010. **24**(1): p. 51-70.
37. Styrkarsdottir, U., et al., *Whole-genome sequencing identifies rare genotypes in COMP and CHADL associated with high risk of hip osteoarthritis.* Nat Genet, 2017. **49**(5): p. 801-805.
38. Lokau, J., et al., *The SNP rs4252548 (R112H) which is associated with reduced human height compromises the stability of IL-11.* Biochim Biophys Acta Mol Cell Res, 2018. **1865**(3): p. 496-506.

Supplementary data

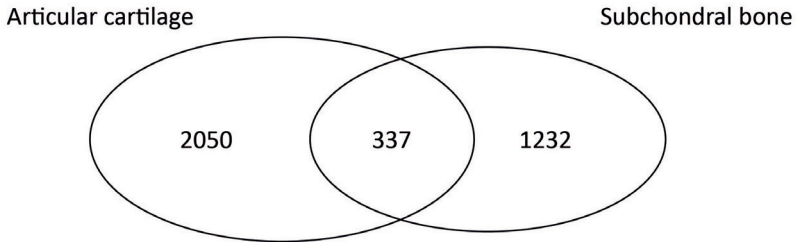
Supplementary figures



Supplementary Figure 1 – Power calculations to determine the sample size of the current study. The parameters used to generate the graph are based on similar analysis on articular cartilage



Supplementary Figure 2 – Silhouette width score showing an optimal number of two clusters.



Supplementary Figure 3 – Venn diagram of differentially expressed genes in the articular cartilage (N=2387) and in the subchondral bone (N=1569).
337 genes were overlapping between cartilage and bone, of which 305 genes show similar direction of effects between cartilage and bone.

Supplementary tables

Supplementary table 1 – Baseline characteristics of subchondral bone samples included in the study

	Total (N=34)	RNAseq - hips (N=6)	RNAseq - knees (N=18)	RT-qPCR - biological (N=10)	RT-qPCR - technical (N=10)
Age (SD)	68,1 (9,5)	67,8 (8,8)	65,7 (8,5)	72,4 (10)	67,6 (7,8)
Females (total)	27 (34)	6 (6)	16 (18)	5 (10)	8 (10)

Supplementary table 2 – Gene expression levels of cartilage and bone markers measured in preserved and lesioned bone and cartilage tissue.
In the statistical analysis cartilage is set as the reference.

		Preserved Cartilage vs. Bone		Lesioned Cartilage vs. Bone	
Marker	**Genes**	**Fold change**	**FDR**	**Fold change**	**FDR**
Cartilage	COL2A1	0.02	7.46E-48	0.01	7.93E-24
Cartilage	COMP	0.01	1.03E-60	0.01	2.76E-47
Cartilage	CRTAC1	0.01	1.64E-112	0.01	1.88E-75
Bone	COL1A1	1.85	1.78E-01	4.31	2.25E-03
Bone	SPP1	8.85	3.54E-19	2.56	3.56E-04
Bone	BGLAP	9.17	5.92E-08	11.19	1.59E-11

Supplementary table 3 (partially) – Significantly differentially expressed genes between lesioned and preserved OA subchondral bone.
Top 50 most significantly differentially expressed genes are shown here, the rest of the table can be found in the online supplement: https://doi.org/10.1002/art.41600

Ensembl gene ID	Gene	P-value	FDR	Log 2 fold change	Fold change
ENSG00000162998	FRZB	2.52E-11	3.99E-07	-0.90	0.53
ENSG00000174469	CNTNAP2	6.16E-09	3.36E-05	1.27	2.42
ENSG00000157103	SLC6A1	6.36E-09	3.36E-05	-0.71	0.61
ENSG00000162105	SHANK2	1.12E-08	4.43E-05	-1.15	0.45
ENSG00000054938	CHRDL2	4.10E-08	1.20E-04	-2.85	0.14
ENSG00000223561	AC005165.1	4.54E-08	1.20E-04	-1.23	0.43
ENSG00000179399	GPC5	7.22E-08	1.27E-04	-1.22	0.43
ENSG00000159307	SCUBE1	6.66E-08	1.27E-04	-0.84	0.56
ENSG00000198918	RPL39	7.12E-08	1.27E-04	0.39	1.31
ENSG00000116285	ERRFI1	8.10E-08	1.28E-04	0.57	1.48
ENSG00000102466	FGF14	1.67E-07	1.89E-04	-1.29	0.41
ENSG00000007314	SCN4A	1.42E-07	1.89E-04	-0.63	0.65
ENSG00000251322	SHANK3	1.61E-07	1.89E-04	-0.51	0.70
ENSG00000144867	SRPRB	1.67E-07	1.89E-04	0.45	1.36
ENSG00000037042	TUBG2	2.74E-07	2.89E-04	-0.36	0.78
ENSG00000196104	SPOCK3	3.44E-07	3.41E-04	-2.07	0.24
ENSG00000169871	TRIM56	4.18E-07	3.90E-04	-0.31	0.81
ENSG00000134014	ELP3	5.24E-07	4.61E-04	0.20	1.15
ENSG00000106511	MEOX2	6.35E-07	5.30E-04	-0.52	0.70
ENSG00000257017	HP	7.30E-07	5.57E-04	1.37	2.59
ENSG00000130158	DOCK6	7.38E-07	5.57E-04	-0.41	0.75
ENSG00000136237	RAPGEF5	8.72E-07	6.28E-04	-0.47	0.72
ENSG00000163884	KLF15	9.79E-07	6.46E-04	-0.67	0.63
ENSG00000204301	NOTCH4	9.58E-07	6.46E-04	-0.41	0.75
ENSG00000234797	RPS3AP6	1.02E-06	6.46E-04	0.25	1.19
ENSG00000002745	WNT16	1.26E-06	6.88E-04	2.12	4.35
ENSG00000064886	CHI3L2	1.22E-06	6.88E-04	1.26	2.40
ENSG00000151025	GPR158	1.17E-06	6.88E-04	1.21	2.31
ENSG00000154783	FGD5	1.21E-06	6.88E-04	-0.35	0.78
ENSG00000130300	PLVAP	1.42E-06	7.33E-04	-0.46	0.73
ENSG00000252835	SCARNA21	1.43E-06	7.33E-04	0.37	1.29
ENSG00000115616	SLC9A2	1.62E-06	8.01E-04	1.53	2.89
ENSG00000232044	SILC1	1.79E-06	8.60E-04	1.09	2.13

Ensembl gene ID	Gene	P-value	FDR	Log 2 fold change	Fold change
ENSG00000112306	RPS12	1.89E-06	8.81E-04	0.23	1.17
ENSG00000128917	DLL4	2.13E-06	9.31E-04	-0.63	0.65
ENSG00000146830	GIGYF1	2.07E-06	9.31E-04	-0.29	0.82
ENSG00000225178	RPSAP58	2.17E-06	9.31E-04	0.28	1.21
ENSG00000123610	TNFAIP6	2.53E-06	1.03E-03	0.95	1.93
ENSG00000173801	JUP	2.48E-06	1.03E-03	-0.51	0.70
ENSG00000148400	NOTCH1	2.67E-06	1.06E-03	-0.53	0.69
ENSG00000089157	RPLP0	2.84E-06	1.10E-03	0.28	1.22
ENSG00000229847	EMX2OS	3.07E-06	1.11E-03	-1.26	0.42
ENSG00000148848	ADAM12	3.02E-06	1.11E-03	0.80	1.75
ENSG00000115128	SF3B6	2.98E-06	1.11E-03	0.25	1.19
ENSG00000149380	P4HA3	3.24E-06	1.12E-03	1.04	2.05
ENSG00000163902	RPN1	3.18E-06	1.12E-03	0.20	1.15
ENSG00000078018	MAP2	3.77E-06	1.27E-03	-0.44	0.74
ENSG00000166426	CRABP1	4.26E-06	1.36E-03	1.43	2.69

Supplementary table 4 (partially) – Gene enrichment analysis on differentially expressed genes (N=1569 genes) in OA subchondral bone.
The five most significant processes are shown here, the rest of the table can be found in the online supplement: https://doi.org/10.1002/art.41600

Category	Term	Count	%	Genes	P-value	FDR
Biological processes	GO:0006614~SRP-dependent cotranslational protein targeting to membrane	33	2.2	RPL26L1, SEC61A1, RPL6, RPLP0, RPL34, RPS13, RPS12, RPL5, RPL23, RPL27, RPS6, RPS24, RPL11, RPS8, SRPRB, RPS3A, RPL7, RPL7A, RPL27A, RPSA, RPS7, RPL4, RPS17, RPL35A, RPS23, RPL37A, RPL12, RPS4X, RPL23A, RPL10A, RPL39, RPL41, RPS18	2.29E-10	4.27E-07
Biological processes	GO:0000184~nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	36	2.4	RPL26L1, SMG6, RPL6, RPLP0, EIF3E, RPL34, RPS13, RPS12, RPL5, RPL23, RPL27, CTIF, NCBP1, RPS6, RPS24, EIF4A3, RPL11, RPS8, RPS3A, RPL7, RPL7A, RPL27A, RPSA, RPS7, RPL4, RPS17, RPL35A, RPS23, RPL37A, RPL12, RPS4X, RPL23A, RPL10A, RPL39, RPL41, RPS18	2.83E-09	5.28E-06
Biological processes	GO:0019083~viral transcription	33	2.2	RPL26L1, RPL6, RPLP0, RPL34, RPS13, RPS12, RPL5, RPL23, NUP214, RPL27, RPS6, RPS24, RPL11, RPS8, RPS3A, RPL7, RPL7A, RPL27A, RPSA, RPS7, RPL4, RPS17, RPL35A, RPS23, POM121, RPL37A, RPL12, RPS4X, RPL23A, RPL10A, RPL39, RPL41, RPS18	4.32E-08	8.06E-05
Biological processes	GO:0006413~translational initiation	36	2.4	RPL26L1, RPL6, RPLP0, EIF3E, RPL34, RPS13, RPS12, RPL5, RPL23, EIF2S2, RPL27, EIF2S1, RPS6, RPS24, RPL11, RPS8, EIF2A, RPS3A, RPL7, RPL7A, EIF3M, RPL27A, RPSA, RPS7, RPL4, RPS17, RPL35A, RPS23, RPL37A, RPL12, RPS4X, RPL23A, RPL10A, RPL39, RPL41, RPS18	1.04E-07	1.95E-04
Biological processes	GO:0018279~protein N-linked glycosylation via asparagine	16	1.1	FUT8, LMAN1, MAGT1, TUSC3, RPN2, ALG5, DAD1, UGGT1, ST6GAL2, ST6GALNAC6, STT3B, RPN1, MGAT2, UBE2J1, OSTC, DDOST	2.95E-06	5.51E-03
Cellular component	GO:0022625~cytosolic large ribosomal subunit	22	1.5	RPL26L1, RPL6, RPLP0, RPL34, RPL5, RPL23, RPL27, RPL11, RPL7L1, RPL7, SURF6, RPL7A, RPL22L1, RPL27A, RPL4, RPL35A, RPL37A, RPL12, RPL23A, RPL10A, RPL39, RPL41	7.73E-07	1.17E-03
Cellular component	GO:0008250~oligosaccharyltransferase complex	8	0.5	MAGT1, TUSC3, RPN2, DAD1, STT3B, RPN1, OSTC, DDOST	5.07E-06	7.66E-03

Supplementary table 5 – Gene enrichment analysis on differentially expressed genes in OA subchondral bone showing an absolute fold change of 2 or higher (N=53 genes)

Category	Term	Count	%	Genes	FDR
Cellular component	GO:0005615~extracellular space	16	32.7	WNT16, CRLF1, CHRDL2, CHI3L2, FAP, IL11, OGN, POSTN, GDF6, CTHRC1, LEP, GPC5, SPOCK3, MSMP, HP, CCL18	1.19E-05
Cellular component	GO:0005578~proteinaceous extracellular matrix	7	14.3	WNT16, OGN, ASPN, POSTN, CTHRC1, GPC5, SPOCK3	4.50E-02

Supplementary table 6 (partially) - Overlapping differentially expressed genes between the subchondral bone and the articular cartilage with similar direction of effect.
Top 50 genes differentially expressed genes in subchondral bone and articular cartilage are shown here, the rest of the table can be found online: https://doi.org/10.1002/art.41600

		Subchondral bone		Articular Cartilage	
Ensembl ID	**Gene name**	**Fold change**	**FDR**	**Fold change**	**FDR**
ENSG00000054938	CHRDL2	0.14	1.20E-04	0.13	7.07E-09
ENSG00000002745	WNT16	4.35	6.88E-04	8.48	1.10E-13
ENSG00000095752	IL11	4.16	2.44E-03	22.80	1.53E-20
ENSG00000196104	SPOCK3	0.24	3.41E-04	0.22	1.56E-09
ENSG00000130294	KIF1A	0.25	1.27E-02	0.37	8.64E-08
ENSG00000006016	CRLF1	2.32	2.86E-02	3.04	2.96E-10
ENSG00000179399	GPC5	0.43	1.27E-04	0.36	1.47E-08
ENSG00000189056	RELN	0.53	2.56E-02	0.22	7.37E-12
ENSG00000123610	TNFAIP6	1.93	1.03E-03	3.58	2.48E-08
ENSG00000151025	GPR158	2.31	6.88E-04	2.73	3.63E-03
ENSG00000259916	AL845331.2	0.46	3.16E-02	0.34	3.50E-02
ENSG00000162998	FRZB	0.53	3.99E-07	0.27	1.87E-09
ENSG00000178752	ERFE	1.87	1.63E-02	3.44	8.82E-12
ENSG00000198729	PPP1R14C	2.19	1.14E-02	2.52	1.33E-11
ENSG00000121005	CRISPLD1	0.51	1.84E-02	0.36	9.29E-06
ENSG00000048540	LMO3	0.58	3.82E-03	0.28	1.67E-05
ENSG00000095777	MYO3A	2.44	1.27E-02	2.25	1.16E-04
ENSG00000256995	AC084816.1	0.38	2.20E-02	0.45	2.20E-05
ENSG00000223561	AC005165.1	0.43	1.20E-04	0.45	5.31E-04
ENSG00000187595	ZNF385C	0.51	3.82E-03	0.43	2.30E-06
ENSG00000106809	OGN	3.43	4.62E-03	2.00	1.02E-03
ENSG00000148344	PTGES	1.64	1.63E-02	3.06	3.61E-12
ENSG00000159307	SCUBE1	0.56	1.27E-04	0.42	2.15E-06
ENSG00000166033	HTRA1	1.73	1.57E-02	2.39	1.65E-11
ENSG00000133110	POSTN	2.04	3.44E-02	2.06	3.20E-02
ENSG00000120149	MSX2	1.64	3.13E-02	2.44	2.45E-05
ENSG00000125144	MT1G	2.16	2.50E-02	1.97	1.72E-04
ENSG00000134198	TSPAN2	1.64	1.51E-02	2.42	1.51E-08
ENSG00000094963	FMO2	0.63	2.56E-02	0.38	2.20E-03
ENSG00000169884	WNT10B	1.49	3.25E-02	3.47	1.52E-06
ENSG00000007314	SCN4A	0.65	1.89E-04	0.38	4.36E-03
ENSG00000141469	SLC14A1	0.53	1.71E-02	0.51	7.05E-06
ENSG00000149380	P4HA3	2.05	1.12E-03	1.84	1.49E-05

		Subchondral bone		Articular Cartilage	
Ensembl ID	**Gene name**	**Fold change**	**FDR**	**Fold change**	**FDR**
ENSG00000148848	ADAM12	1.75	1.11E-03	1.98	1.85E-04
ENSG00000263155	MYZAP	0.60	1.55E-02	0.47	3.56E-04
ENSG00000089685	BIRC5	1.59	3.77E-02	2.30	2.12E-03
ENSG00000006327	TNFRSF12A	1.50	2.31E-02	2.68	1.14E-08
ENSG00000100473	COCH	1.46	4.21E-02	3.30	1.01E-08
ENSG00000102466	FGF14	0.41	1.89E-04	0.58	2.01E-04
ENSG00000171017	LRRC8E	1.72	3.89E-02	1.99	1.11E-04
ENSG00000280339	AP001528.3	0.66	2.65E-02	0.38	1.28E-06
ENSG00000167037	SGSM1	0.63	2.89E-03	0.47	1.30E-06
ENSG00000196352	CD55	1.46	2.48E-02	2.96	1.05E-14
ENSG00000116147	TNR	0.65	1.98E-02	0.44	1.08E-03
ENSG00000142149	HUNK	1.71	4.91E-02	1.94	1.04E-03
ENSG00000140538	NTRK3	0.70	3.56E-03	0.31	2.64E-05
ENSG00000106819	ASPN	3.17	3.56E-03	1.65	3.04E-02
ENSG00000124440	HIF3A	0.49	2.07E-03	0.58	2.72E-02

Supplementary table 7 – Overlapping differentially expressed genes between the subchondral bone and the articular cartilage with opposite direction of effect.

		Subchondral bone		Articular Cartilage	
Ensembl ID	**Gene name**	**Fold change**	**FDR**	**Fold change**	**FDR**
ENSG00000074181	NOTCH3	0.70	1.44E-03	2.03	1.13E-03
ENSG00000081277	PKP1	0.57	5.32E-03	1.62	4.84E-03
ENSG00000082175	PGR	0.73	2.79E-02	1.49	2.02E-02
ENSG00000088387	DOCK9	0.88	2.90E-02	1.32	2.17E-02
ENSG00000100234	TIMP3	0.70	4.39E-03	1.54	9.97E-06
ENSG00000103528	SYT17	0.80	4.66E-02	1.21	2.56E-02
ENSG00000109846	CRYAB	0.67	2.83E-02	1.32	6.16E-03
ENSG00000110092	CCND1	0.84	3.29E-02	1.63	1.14E-04
ENSG00000119185	ITGB1BP1	0.81	2.45E-02	1.22	5.71E-04
ENSG00000120278	PLEKHG1	0.85	4.29E-02	1.77	7.93E-04
ENSG00000120318	ARAP3	0.85	3.56E-03	1.34	4.38E-03
ENSG00000144476	ACKR3	0.76	3.99E-02	1.34	7.96E-03
ENSG00000145911	N4BP3	0.68	1.92E-03	2.18	3.27E-03
ENSG00000146674	IGFBP3	0.78	2.63E-02	2.65	1.12E-07
ENSG00000156453	PCDH1	0.82	2.19E-02	1.91	4.02E-05
ENSG00000157510	AFAP1L1	0.80	1.93E-02	2.08	9.44E-04
ENSG00000157617	C2CD2	0.74	2.45E-02	1.28	1.98E-03
ENSG00000158258	CLSTN2	0.66	6.38E-03	1.83	1.40E-02
ENSG00000173210	ABLIM3	0.76	3.70E-03	1.98	9.31E-06
ENSG00000173599	PC	0.83	3.60E-02	1.25	3.51E-03
ENSG00000197183	NOL4L	0.84	2.56E-02	1.25	1.02E-03
ENSG00000198517	MAFK	0.86	2.43E-02	1.35	4.36E-03
ENSG00000198742	SMURF1	0.90	4.93E-02	1.55	3.32E-07
ENSG00000205336	ADGRG1	0.78	3.59E-02	1.73	1.39E-03
ENSG00000052850	ALX4	2.08	2.30E-03	0.55	2.75E-02
ENSG00000099284	H2AFY2	1.23	2.32E-02	0.85	2.07E-02
ENSG00000106066	CPVL	1.26	8.94E-03	0.53	9.16E-03
ENSG00000144649	GASK1A	1.48	1.49E-02	0.54	1.86E-03
ENSG00000165973	NELL1	1.74	3.11E-02	0.47	2.05E-02
ENSG00000182326	C1S	1.29	1.37E-02	0.77	7.74E-03
ENSG00000182853	VMO1	1.58	4.38E-02	0.53	1.63E-03
ENSG00000264672	SEPT4-AS1	1.44	2.30E-02	0.60	1.94E-02

Supplementary table 8 – Gene enrichment analysis on overlapping genes with similar direction of effect between the subchondral bone and the articular cartilage (N= 305 genes).

Category	Term	Count	%	Genes	FDR
Cellular Component	GO:0005615~extracellular space	37	12	WNT16, CRLF1, VCAN, DKK3, CHRDL2, FAP, DLG3, WNT11, IL11, CCN4, OGN, TPI1, FRMD4B, SPP1, TNFSF11, TNFAIP6, OMD, POSTN, CD63, GGH, LUM, MANF, LGI4, SEMA3D, GDF6, SCUBE1, FRZB, COL6A3, FSTL1, HTRA1, GPRC5B, ERFE, GPC5, RELN, SPOCK3, S100A4, GPX3	4.42E-03
Cellular Component	GO:0005576~extracellular region	36	12	WNT16, CRLF1, VCAN, DKK3, WNT11, IL11, FGF14, OGN, TNR, SPP1, TNFSF11, CRISPLD1, OMD, CALU, FGF13, SPATA6, PDZD2, NGF, FST, LUM, ADAM12, PAMR1, CRIM1, LGI4, GDF6, FRZB, COL6A3, FSTL1, HTRA1, THBS3, HTRA3, ERFE, GPC5, PLAC9, CD55, GPX3	4.56E-03
Cellular Component	GO:0005578~proteinaceous extracellular matrix	17	6	WNT16, VCAN, WNT11, CHADL, COCH, CCN4, OGN, ASPN, TNR, OMD, POSTN, LUM, ADAMTS17, COL6A3, GPC5, RELN, SPOCK3	7.98E-03

Supplementary table 9 (partially) – Significantly differentially expressed genes in OA knee subchondral bone.
Top 50 most significantly differentially expressed genes in knee subchondral bone are shown here, the rest of the table can be found online: https://doi.org/10.1002/art.41600

Ensembl gene ID	Gene	P-value	FDR	Log 2 fold change	Fold change
ENSG00000162998	FRZB	3.07E-12	5.13E-08	-1.05	0.48
ENSG00000174469	CNTNAP2	4.05E-10	3.38E-06	1.54	2.90
ENSG00000054938	CHRDL2	7.41E-10	4.13E-06	-3.47	0.09
ENSG00000116285	ERRFI1	1.61E-09	6.70E-06	0.68	1.60
ENSG00000157103	SLC6A1	3.77E-09	1.16E-05	-0.76	0.59
ENSG00000178445	GLDC	4.16E-09	1.16E-05	1.78	3.44
ENSG00000113594	LIFR	1.96E-08	4.68E-05	0.43	1.34
ENSG00000179399	GPC5	3.64E-08	7.59E-05	-1.36	0.39
ENSG00000198918	RPL39	4.15E-08	7.71E-05	0.42	1.34
ENSG00000115616	SLC9A2	4.72E-08	7.88E-05	1.75	3.35
ENSG00000102466	FGF14	5.51E-08	7.97E-05	-1.41	0.38
ENSG00000151025	GPR158	5.73E-08	7.97E-05	1.34	2.54
ENSG00000223561	AC005165.1	7.31E-08	9.39E-05	-1.31	0.40
ENSG00000257017	HP	9.28E-08	1.11E-04	1.79	3.46
ENSG00000154783	FGD5	1.17E-07	1.23E-04	-0.40	0.76
ENSG00000145934	TENM2	1.22E-07	1.23E-04	0.72	1.64
ENSG00000130158	DOCK6	1.26E-07	1.23E-04	-0.45	0.73
ENSG00000229847	EMX2OS	1.45E-07	1.34E-04	-1.52	0.35
ENSG00000106511	MEOX2	1.56E-07	1.34E-04	-0.51	0.70
ENSG00000168685	IL7R	1.64E-07	1.34E-04	1.07	2.10
ENSG00000171517	LPAR3	1.75E-07	1.34E-04	1.11	2.15
ENSG00000175161	CADM2	1.81E-07	1.34E-04	-1.46	0.36
ENSG00000002745	WNT16	1.85E-07	1.34E-04	2.39	5.23
ENSG00000144057	ST6GAL2	2.31E-07	1.61E-04	2.29	4.88
ENSG00000249306	LINC01411	2.54E-07	1.70E-04	2.65	6.27
ENSG00000104435	STMN2	2.74E-07	1.76E-04	4.52	23.00
ENSG00000159307	SCUBE1	3.10E-07	1.92E-04	-0.92	0.53
ENSG00000144867	SRPRB	3.95E-07	2.36E-04	0.51	1.43
ENSG00000187244	BCAM	4.10E-07	2.36E-04	-0.61	0.66
ENSG00000169871	TRIM56	4.26E-07	2.37E-04	-0.36	0.78
ENSG00000110237	ARHGEF17	6.59E-07	3.55E-04	-0.39	0.76
ENSG00000123610	TNFAIP6	6.95E-07	3.63E-04	1.17	2.26
ENSG00000148400	NOTCH1	8.50E-07	4.30E-04	-0.65	0.64

Ensembl gene ID	Gene	P-value	FDR	Log 2 fold change	Fold change
ENSG00000232044	SILC1	9.27E-07	4.55E-04	1.19	2.28
ENSG00000138829	FBN2	1.13E-06	5.37E-04	1.01	2.02
ENSG00000146830	GIGYF1	1.18E-06	5.49E-04	-0.33	0.80
ENSG00000074181	NOTCH3	1.27E-06	5.74E-04	-0.61	0.66
ENSG00000150938	CRIM1	1.42E-06	6.22E-04	-0.55	0.68
ENSG00000196104	SPOCK3	1.81E-06	7.25E-04	-2.43	0.19
ENSG00000159200	RCAN1	1.81E-06	7.25E-04	0.57	1.49
ENSG00000171714	ANO5	1.82E-06	7.25E-04	0.92	1.89
ENSG00000134014	ELP3	1.82E-06	7.25E-04	0.22	1.17
ENSG00000107719	PALD1	2.21E-06	8.59E-04	-0.34	0.79
ENSG00000125869	LAMP5	2.31E-06	8.63E-04	0.92	1.90
ENSG00000066056	TIE1	2.33E-06	8.63E-04	-0.40	0.76
ENSG00000162105	SHANK2	2.60E-06	9.45E-04	-1.10	0.47
ENSG00000166426	CRABP1	2.82E-06	9.84E-04	1.54	2.91
ENSG00000140464	PML	2.83E-06	9.84E-04	-0.37	0.78
ENSG00000172986	GXYLT2	3.32E-06	1.10E-03	0.69	1.61

Supplementary table 10 - Significant differentially expressed genes exclusive for knee OA subchondral bone.
Top 50 most significantly differentially expressed genes exclusively for knee subchondral bone are shown here, the rest of the table can be found online: https://doi.org/10.1002/art.41600

Ensembl gene ID	Gene	P-value	FDR	Log 2 fold change	Fold change
ENSG00000249306	LINC01411	2.54E-07	1.70E-04	2.65	6.27
ENSG00000072041	SLC6A15	4.09E-06	1.22E-03	-1.73	0.30
ENSG00000113263	ITK	9.38E-06	1.76E-03	0.58	1.49
ENSG00000196787	HIST1H2AG	9.75E-06	1.76E-03	0.58	1.50
ENSG00000275221	HIST1H2AK	1.18E-05	1.90E-03	0.58	1.49
ENSG00000101057	MYBL2	1.20E-05	1.91E-03	1.07	2.10
ENSG00000122966	CIT	1.24E-05	1.93E-03	0.51	1.43
ENSG00000274997	HIST1H2AH	1.37E-05	1.98E-03	0.55	1.47
ENSG00000278272	HIST1H3C	1.64E-05	2.19E-03	0.84	1.79
ENSG00000138160	KIF11	1.89E-05	2.39E-03	0.68	1.60
ENSG00000100593	ISM2	1.91E-05	2.39E-03	1.77	3.40
ENSG00000162739	SLAMF6	2.05E-05	2.47E-03	0.79	1.73
ENSG00000169679	BUB1	2.50E-05	2.81E-03	0.79	1.73
ENSG00000090382	LYZ	2.56E-05	2.82E-03	0.64	1.56
ENSG00000277224	HIST1H2BF	2.59E-05	2.83E-03	0.58	1.49
ENSG00000253141	AC008632.1	2.76E-05	2.91E-03	-1.68	0.31
ENSG00000019505	SYT13	2.81E-05	2.92E-03	2.33	5.04
ENSG00000185730	ZNF696	2.81E-05	2.92E-03	-0.39	0.77
ENSG00000136167	LCP1	3.09E-05	2.98E-03	0.45	1.37
ENSG00000158481	CD1C	3.08E-05	2.98E-03	0.69	1.61
ENSG00000274267	HIST1H3B	3.20E-05	3.05E-03	0.75	1.69
ENSG00000205268	PDE7A	4.93E-05	4.07E-03	0.39	1.31
ENSG00000184357	HIST1H1B	5.70E-05	4.40E-03	0.61	1.53
ENSG00000171388	APLN	5.81E-05	4.43E-03	-0.51	0.70
ENSG00000126787	DLGAP5	5.91E-05	4.44E-03	1.23	2.35
ENSG00000276410	HIST1H2BB	7.06E-05	4.89E-03	0.57	1.49
ENSG00000117724	CENPF	7.62E-05	5.07E-03	0.71	1.64
ENSG00000125354	SEPT6	7.86E-05	5.10E-03	0.30	1.23
ENSG00000197635	DPP4	8.10E-05	5.20E-03	0.76	1.69
ENSG00000131747	TOP2A	8.28E-05	5.28E-03	0.77	1.71
ENSG00000130812	ANGPTL6	9.02E-05	5.54E-03	1.34	2.53
ENSG00000131475	VPS25	9.78E-05	5.90E-03	0.26	1.19
ENSG00000197153	HIST1H3J	1.15E-04	6.24E-03	0.94	1.91

Ensembl gene ID	Gene	P-value	FDR	Log 2 fold change	Fold change
ENSG00000169385	RNASE2	1.18E-04	6.35E-03	1.22	2.33
ENSG00000273703	HIST1H2BM	1.21E-04	6.48E-03	0.91	1.88
ENSG00000105639	JAK3	1.24E-04	6.57E-03	0.54	1.45
ENSG00000049540	ELN	1.40E-04	7.03E-03	-0.55	0.68
ENSG00000140157	NIPA2	1.59E-04	7.49E-03	0.33	1.26
ENSG00000273983	HIST1H3G	1.60E-04	7.49E-03	0.88	1.84
ENSG00000172575	RASGRP1	1.64E-04	7.54E-03	0.60	1.51
ENSG00000197057	DTHD1	1.68E-04	7.55E-03	0.97	1.95
ENSG00000103145	HCFC1R1	1.78E-04	7.74E-03	0.30	1.23
ENSG00000085265	FCN1	1.85E-04	7.75E-03	0.55	1.47
ENSG00000128641	MYO1B	1.84E-04	7.75E-03	0.39	1.31
ENSG00000139734	DIAPH3	1.82E-04	7.75E-03	1.05	2.07
ENSG00000118193	KIF14	2.03E-04	8.28E-03	0.73	1.66
ENSG00000266524	GDF10	2.11E-04	8.40E-03	-0.79	0.58
ENSG00000076685	NT5C2	2.23E-04	8.62E-03	0.19	1.14
ENSG00000182566	CLEC4G	2.22E-04	8.62E-03	0.99	1.99

Supplementary table 11 (partially) – Gene enrichment analysis on genes exclusively differentially expressed in knee OA subchondral bone samples (N=509 genes).
The five most significant processes are shown here, the rest of the table can be found in the online supplement: https://doi.org/10.1002/art.41600

Category	Term	Count	%	Genes	FDR
Biological process	GO:0006334~nucleosome assembly	15	3.0	HIST1H1B, H1FX, HIST1H2BL, HIST1H3J, HIST1H4K, HIST1H2BM, HIST1H3G, HIST1H3B, HIST1H2BH, HIST1H2BB, HIST1H2BF, HIST1H3F, HIST1H3C, HIST1H2BI, HIST1H3H	2.91E-03
Biological process	GO:0060968~regulation of gene silencing	6	1.2	HIST1H3J, HIST1H3G, HIST1H3B, HIST1H3F, HIST1H3C, HIST1H3H	1.90E-02
Cellular component	GO:0000786~nucleosome	20	4.1	HIST1H1B, H1FX, HIST1H2BL, HIST1H2AG, HIST1H3J, HIST1H4K, HIST1H2BM, HIST1H3G, HIST1H3B, HIST1H2AH, HIST1H2AK, HIST1H2BH, HIST1H2AJ, HIST1H2BB, HIST1H2BF, HIST1H3F, HIST1H3C, HIST1H2AB, HIST1H2BI, HIST1H3H	1.81E-09
Cellular component	GO:0000788~nuclear nucleosome	12	2.4	HIST1H2BL, HIST1H3J, HIST1H2BM, HIST1H3G, HIST1H3B, HIST1H2BH, HIST1H2BB, HIST1H2BF, HIST1H3F, HIST1H3C, HIST1H2BI, HIST1H3H	4.37E-06
Reactome pathway	R-HSA-2299718:Condensation of Prophase Chromosomes	17	3.5	PLK1, CDK1, HIST1H2BL, HIST1H3J, HIST1H4K, HIST1H2BM, HIST1H3G, HIST1H3B, HIST1H2BH, HIST1H2AJ, HIST1H2BB, HIST1H2BF, HIST1H3F, HIST1H3C, HIST1H2AB, HIST1H2BI, HIST1H3H	2.52E-07

Supplementary table 12 - Differentially expressed genes in hip samples selected on their nominal p-value.

Ensembl gene ID	Gene Name	P-value	Log 2 fold change	Fold change
ENSG00000064886	CHI3L2	5.08E-05	1.047	2.07
ENSG00000120738	EGR1	1.17E-04	-1.367	0.39
ENSG00000106809	OGN	5.06E-04	2.646	6.26
ENSG00000279407	AC007191.1	4.39E-03	-1.292	0.41
ENSG00000143512	HHIPL2	7.41E-03	1.515	2.86
ENSG00000131459	GFPT2	7.63E-03	1.199	2.30
ENSG00000169884	WNT10B	8.43E-03	1.236	2.35
ENSG00000139219	COL2A1	8.72E-03	2.514	5.71
ENSG00000162105	SHANK2	1.14E-02	-1.350	0.39
ENSG00000100302	RASD2	1.18E-02	-1.602	0.33
ENSG00000280800	FP671120.4	1.34E-02	-1.827	0.28
ENSG00000167094	TTC16	1.35E-02	-1.306	0.40
ENSG00000180389	ATP5F1EP2	1.60E-02	1.131	2.19
ENSG00000260105	AOC4P	2.09E-02	-1.706	0.31
ENSG00000006016	CRLF1	2.27E-02	1.232	2.35
ENSG00000279662	AC131649.2	2.72E-02	-1.192	0.44
ENSG00000149380	P4HA3	2.95E-02	0.997	2.00
ENSG00000261026	AC105046.1	3.09E-02	-1.338	0.40
ENSG00000004948	CALCR	3.12E-02	-1.223	0.43
ENSG00000125740	FOSB	3.32E-02	-1.696	0.31
ENSG00000275765	AC091982.3	3.42E-02	1.048	2.07
ENSG00000283199	C13orf46	3.51E-02	-1.227	0.43
ENSG00000267653	AC002546.1	3.63E-02	-1.214	0.43
ENSG00000106819	ASPN	3.65E-02	1.770	3.41
ENSG00000233013	FAM157B	3.73E-02	-1.144	0.45
ENSG00000116147	TNR	3.75E-02	-1.052	0.48
ENSG00000139292	LGR5	4.27E-02	-1.454	0.36
ENSG00000095752	IL11	4.43E-02	2.253	4.77
ENSG00000253132	IGHV3-62	4.79E-02	1.064	2.09

Supplementary table 13 - Significant differentially expressed genes exclusive for hip OA subchondral bone

Ensembl gene ID	Gene Name	P-value	Log 2 Fold Change	Fold Change
ENSG00000004948	CALCR	3.12E-02	-1.22	0.43
ENSG00000120738	EGR1	1.17E-04	-1.37	0.39
ENSG00000125740	FOSB	3.32E-02	-1.70	0.31
ENSG00000139292	LGR5	4.27E-02	-1.45	0.36
ENSG00000167094	TTC16	1.35E-02	-1.31	0.40
ENSG00000233013	FAM157B	3.73E-02	-1.14	0.45
ENSG00000260105	AOC4P	2.09E-02	-1.71	0.31
ENSG00000261026	AC105046.1	3.09E-02	-1.34	0.40
ENSG00000279407	AC007191.1	4.39E-03	-1.29	0.41
ENSG00000279662	AC131649.2	2.72E-02	-1.19	0.44
ENSG00000280800	FP671120.4	1.34E-02	-1.83	0.28
ENSG00000283199	C13orf46	3.51E-02	-1.23	0.43
ENSG00000131459	GFPT2	7.63E-03	1.20	2.30
ENSG00000139219	COL2A1	8.72E-03	2.51	5.71
ENSG00000143512	HHIPL2	7.41E-03	1.52	2.86
ENSG00000180389	ATP5F1EP2	1.60E-02	1.13	2.19
ENSG00000253132	IGHV3-62	4.79E-02	1.06	2.09
ENSG00000275765	AC091982.3	3.42E-02	1.05	2.07

Supplementary table 14 - Validation and replication of the RNAseq findings. The preserved samples are set as the reference.

	RNAseq (N=18 samples)			Validation (N=10 technial replicates)			Replication (N=10 biological replicates)		
Genes	**β**	**FC**	**FDR**	**β**	**FC**	**Pval**	**β**	**FC**	**Pval**
FRZB	-6.97	0.48	5.13E-08	-1.82	0.28	2.00E-06	-2.27	0.27	4.10E-05
CHRDL2	-6.16	0.09	4.13E-06	-3.46	0.08	1.00E-03	-3.93	0.10	2.00E-03
POSTN	3.89	2.55	5.99E-03	2.32	5.01	2.93E-04	2.52	3.37	5.00E-04
ASPN	3.52	3.10	1.24E-02	2.33	5.04	1.40E-02	2.21	2.34	1.00E-03
CNTNAP2	6.25	2.90	3.38E-06	1.38	2.60	1.60E-02	1.17	2.14	3.10E-02
STMN2	5.14	23.0	1.76E-04	3.04	7.86	1.20E-02	4.52	9.33	5.77E-07