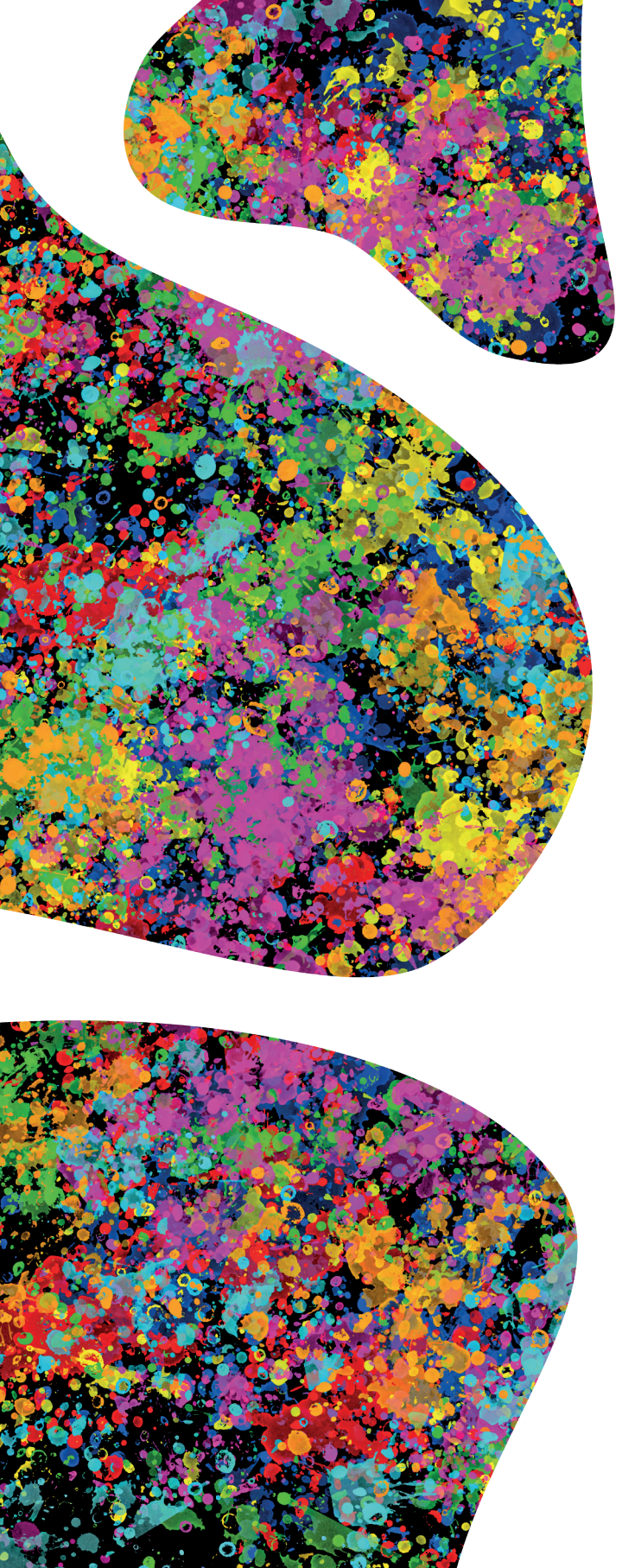
The bone and cartilage interplay in osteoarthritis: key to effective treatment strategy

Tuerlings, M.



CHAPTER 7

Exploring the therapeutic effect of IL11 on lesioned OA human osteochondral explants

Margo Tuerlings[1], Ilja Boone[1], Janneke E. Simon[1], Maurice W. de Haan[1], Evelyn Houtman[1], H. Eka D. Suchiman[1], Robert J.P. van der Wal[2], Rob G.H.H. Nelissen[2], Rachid Mahdad[3], Yolande F.M. Ramos[1], Ingrid Meulenbelt[1]

[1] Dept. of Biomedical Data Sciences, Leiden University Medical Center, Leiden, The Netherlands.
[2] Center for proteomics and metabolomics , Leiden University Medical Center, Leiden, The Netherlands
[3] Dept. Orthopaedics, Alrijne hospital, Leiderdorp, The Netherlands

Abstract

Objective: To explore osteoarthritis (OA) risk gene IL11 co-expression profiles in our previously reported RNA-sequencing datasets of OA articular cartilage and subchondral bone and investigate the potential therapeutic effect of hrIL11 in a biomimetic aged human osteochondral explant model of OA.

Methods: We used RNA-sequencing datasets of macroscopically preserved and lesioned OA articular cartilage (N=35 patients) and subchondral bone (N=24 patients). Spearman correlations were calculated between IL11 expression levels and genes expressed in cartilage (N=20048 genes) or subchondral bone (N=15809 genes). Osteochondral explants were isolated from macroscopically lesioned areas of the joint and were kept in culture for two weeks, with or without exposure to 200ng/ml hrIL11.

Results: We identified more genes being correlated in the lesioned (N=203 and N=198, respectively) compared to preserved (N=106 and N=0, respectively) articular cartilage and subchondral bone. The genes correlated to *IL11* in lesioned cartilage and bone were significantly enriched for processes regarding extracellular space and endoplasmic reticulum, respectively. Exposure of *ex vivo* osteochondral explants to hrIL11 showed minimal effects. In articular cartilage we only observed significant upregulation of *SPP1* and downregulation of *WNT16*, together suggesting a more hypertrophic chondrocyte phenotype upon hrIL11 exposure. In the underlying subchondral bone we only observed significant downregulation of *PTGES* and *IL11RA*, suggesting reduced osteoclast activity. Notably, we observed a different response between patients in terms of intrinsic *IL11* expression levels upon exposure to hrIL11.

Conclusion: The current study shows the importance of functionally investigating OA risk genes, as we here showed that treating the whole joint with hrIL11 as suggested does not necessarily have a beneficial outcome. Based on our results, treatment of OA articular cartilage with hrIL11 shows unbeneficial effects, while treatment of OA subchondral bone with hrIL11 might be positive for both subchondral bone and articular cartilage.

Introduction

Osteoarthritis (OA) is an age-related joint disease in which progressive degeneration of articular cartilage and remodeling of subchondral bone are seen. Therapeutic strategies mainly consist of pain relief treatment, often leading to total joint replacement surgery at end-stage OA. To allow development of new therapeutic strategies, identification of key determinants in OA onset and progression is essential. To discover such key determinants, genome-wide association studies (GWAS) have focused on identification of robust single nucleotide polymorphisms (SNPs) significantly conferring risk to OA [1-4]. In a large genome-wide meta-analysis including data of Icelandic and UK OA patients, rs4252548-T was identified, a SNP in the coding region of interleukin 11 (IL11), being associated to OA [4]. Most recently, this SNP was confirmed in the largest genome-wide meta-analysis so far, including individuals from 9 populations [1]. The identified risk allele, rs4252548-T, is a missense mutation (p.Arg112His) resulting in a thermally unstable protein. As such, it is hypothesized that rs4252548-T confers risk to OA via reduced availability of IL11 protein, whereas, administration of human recombinant IL11 (hrIL11) protein, an approved drug for thrombocytopenia [5], was put forward as a potential therapeutic strategy for OA. Counterintuitively, however, *IL11* gene expression is among the highest upregulated genes both in macroscopically lesioned articular cartilage [6] and subchondral bone [7] compared to preserved tissue. Such high potency of joint tissue cells to upregulate *IL11* is not directly brought into line with the effect of the OA risk missense mutation, unless translation to IL11 protein or receptor signaling with OA is abrogated.

IL11, for that matter, is a member of the interleukin-6 (IL6) cytokine family and can signal via binding to a specific heterodimeric membrane bound complex containing IL11RA and GP130, known as classic signaling, or via binding to soluble IL11RA and a dimeric membrane bound complex of GP130, known as trans signaling [8, 9]. While GP130 is ubiquitously expressed across different cell types, IL11RA is expressed by specific cell types including chondrocytes, osteoblasts, osteocytes, and osteoclasts, indicating an essential, as of yet unclear, role for IL11 in these joint tissues [10-12]. Deletion of IL11 signaling in mice by knocking out IL11RA resulted in increased trabecular bone mass and reduced osteoclast differentiation [13]. Moreover, administration of IL11 in a rheumatoid arthritis murine model resulted in decreased level of synovitis, suggesting IL11 also has anti-inflammatory effects [14].

To obtain more insight into the *IL11* co-expression profiles in articular cartilage and subchondral bone, we used our previously reported RNA-sequencing (RNA-seq) datasets to create co-expression networks [6, 7]. Moreover, to investigate the potential therapeutic effect of hrIL11 on articular cartilage and subchondral bone we exposed

our previously established biomimetic aged human osteochondral explant model of OA to hrIL11 [15].

Methods

Sample characteristics

RNA-sequencing data of macroscopically preserved and lesioned OA articular cartilage (N=35 patients, RAAK-study) and OA subchondral bone (N=24 patients, RAAK-study) was included in the current study. Moreover, preserved and lesioned osteochondral explants were isolated from knee joints of 8 additional patients. Patients characteristics are shown in **Supplementary Table 1**. Classification of macroscopically preserved and lesioned areas of the joint was done as described previously [14]. Ethical approval for the RAAK study was supplied by the medical ethics committee of the Leiden University Medical Centre (P08.239/P19.013).

RNA-sequencing

RNA was isolated from the articular cartilage using Qiagen RNeasy Mini Kit (Qiagen, GmbH, Hilden, Germany). 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 [15] against GRCh38 using default parameters. Read abundances per sample was estimated using HTSeq count v0.11.1 [16]. Only uniquely mapping reads were used for estimating expression. The quality of the raw reads for RNA-sequencing was checked using MultiQC v1.7. [17]. The DESeq2 package [18] was used to normalize the RNA-seq data, as a variance-stabilizing transformation was performed. Data of subchondral bone is available at the European Genome-Phenome Archive (EGAS00001004476) and data of articular cartilage is available at ArrayExpress (E-MTAB-7313).

IL11 co-expression

Spearman correlations were calculated between *IL11* and genes expressed in articular cartilage (N=20048 genes) or subchondral bone (N=15809 genes) using R package Hmisc v.4.2-0. Benjamini-Hochberg method was used to correct for multiple testing. Genes were considered significantly correlating with $|\rho| > 0.6$ and False Discovery Rate (FDR) < 0.05.

Osteochondral explants

Osteochondral explants were isolated from macroscopically preserved and lesioned areas of OA knee joints within 3 hours of joint replacement surgery. Osteochondral

explants containing both subchondral bone and articular cartilage (diameter = 8mm) were cultured as described previously [19]. In short, explants were washed in sterile PBS and taken into culture in chondrogenic differentiation medium (CDM) in a 5% CO_2 incubator at 37°C. On day 3 of culture, hrIL11 protein was added to the culture medium (200ng/ml, PeproTech). The medium containing hrIL11 was refreshed on day 6 and day 10 of culture. After a total culture period of 14 days, cartilage and bone were harvested separately, snap-frozen in liquid nitrogen, and stored in the freezer (-80°C) for further analysis.

RT-qPCR

Articular cartilage and subchondral bone were pulverized separately and homogenized using TRIzol reagent (Invitrogen). RNA was extracted from the samples using RNeasy Mini Kit (Qiagen) and cDNA synthesis was performed using the First Strand cDNA Synthesis Kit (Roche Applied Science) according to manufacturer's protocols. Subsequently, RT-qPCR was performed with the Biomark™ 96.96 Dynamic Arrays (Fluidigm) according to the manufacturer's protocol. Additional RT-qPCR was performed with QuantStudio 6 Real-Time PCR system (Applied Biosystems) using Fast Start SYBR Green Master mix (Roche Applied Science). Gene expression levels were corrected for housekeeping gene *SDHA.* Fold changes were calculated using the $2^{-\Delta\Delta CT}$ method. All values were calculated relative to the control group. Paired generalized estimating equations were applied using SPSS version 25.

(Immuno-) histochemistry

Explants were fixed in 4% formaldehyde overnight, decalcified using EDTA (12.5%, pH=7.4) and embedded in paraffin. Tissue sections were made of 5 um. Subsequently, slides were deparaffinated and rehydrated with Histoclear and ethanol (100-50%). Sections were stained with haematoxylin and eosin (HE staining), toloidine blue (Sigma-Aldrich), or antibody staining was performed. For antibody staining endogenous peroxidase activity was blocked by MeOH/0.3% H_2O_2. Subsequently, antigen retrieval was performed with Proteinase K (25ug/ml) followed by hyaluronidase (5mg/mL). Sections were blocked with 5% non-fat dry milk in PBS and incubated overnight at 4°C with the primary antibody (anti-IL11 Rabbit, 1:50, Thermo Fischer Scientific). The next day, the sections were incubated with Powervision-Poly/HRP (ImmunoLogic), followed by incubation with DAB (Sigma). Sections were dehydrated with ethanol (50-100%) and Histoclear and mounted with Pertex.

Results

IL11 co-expression in articular cartilage and subchondral bone

To identify genes that are regulated by or co-expressed with *IL11* with ongoing

OA, we first performed Spearman correlations between expression levels of *IL11* and genes expressed in articular cartilage (N=20048 genes). As shown in **Figure 1A** and **Figure 1B**, 106 genes were significantly correlating to *IL11* in preserved OA articular cartilage, while 203 genes were correlated to *IL11* in lesioned OA articular cartilage (**Supplementary Table 2**). Only 21 genes were correlated to *IL11* in both preserved and lesioned articular cartilage. This relatively small overlap in correlating genes suggests distinct functions for *IL11* between preserved and lesioned cartilage. However, upon gene enrichment analysis, we identified similar processes for preserved and lesioned cartilage, including extracellular space (GO:0005615) and extracellular region (GO:0005576), characterized by different genes (**Supplementary Table 3**). Among the overlapping genes we identified *PLAUR* ($\rho_{preserved}$=0.60 and $\rho_{lesioned}$=0.77) and *CLCF1* ($\rho_{preserved}$=0.64 and $\rho_{lesioned}$=0.75), both genes showing higher correlations in lesioned articular cartilage. With respect to IL11 receptors, we did not find significant correlations between *IL11* and *IL11RA* and *gp130* expression levels in either preserved

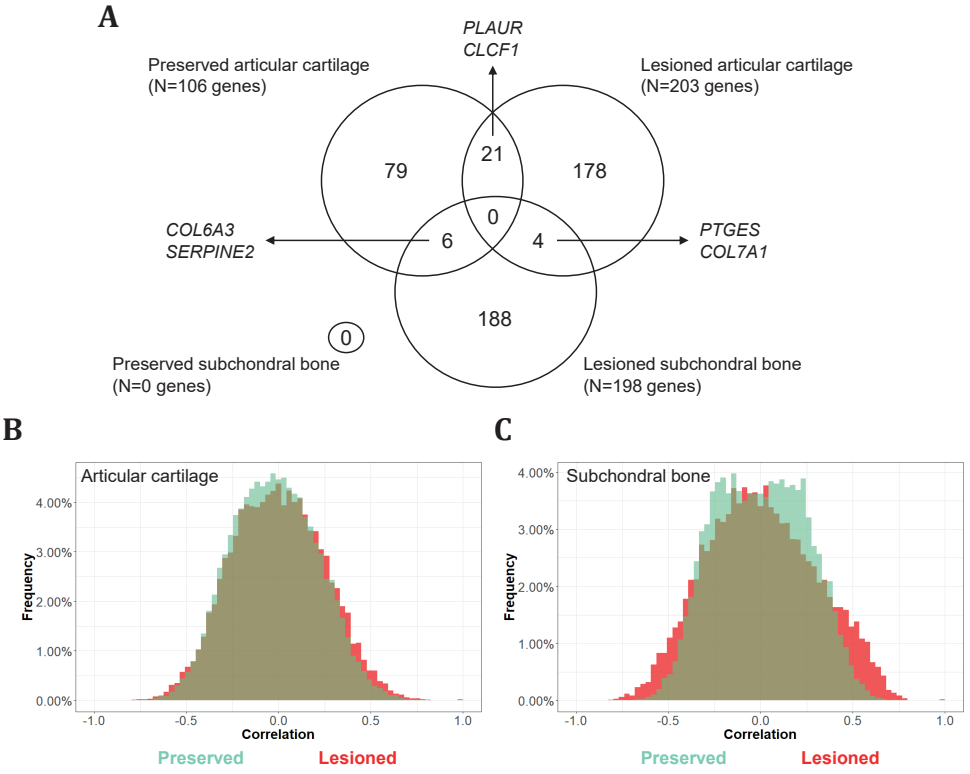


Figure 1 – Spearman correlations between expression levels IL11 and genes expressed in articular cartilage or subchondral bone.
(A) Overlap in significantly correlating genes. Genes with |ρ|>0.6 and FDR<0.05 are considered significantly correlating. (B) Distribution of correlations in subchondral bone. (C) Distribution of correlations in articular cartilage.

cartilage (ρ=0.04 and ρ=-0.21, respectively) or lesioned cartilage (ρ=0.06 and ρ=-0.17, respectively) (**Supplementary Table 4**).

Upon performing Spearman correlations between expression levels of *IL11* and genes expressed in subchondral bone (N=15809 genes) we did not observe any correlations between *IL11* and genes expressed in preserved subchondral bone. In lesioned subchondral bone, we identified 198 genes that significantly correlated to *IL11* expression levels (**Figure 1A** and **Figure 1C, Supplementary Table 5**). Among the highest correlations we found *ELOVL5* (ρ=-0.88) and *WNT16* (ρ=0.81). Gene enrichment analysis including these 198 genes showed significant enrichment for endoplasmic reticulum (GO:0005783) and golgi apparatus (GO:005794) (**Supplementary Table 6**). Moreover, as shown in **Figure 1A,** of these 198 genes correlating to *IL11* in lesioned subchondral bone, 6 genes were also correlating to *IL11* in preserved articular cartilage, including *COL6A3* and *SERPINE2*, and 4 genes were also correlating to *IL11* in lesioned articular cartilage. The increased number of significant correlations in lesioned compared to preserved cartilage and bone tissue suggest that *IL11* plays a role mainly in lesioned OA tissue. With respect to IL11 receptors in subchondral bone, we did not find significant correlations between *IL11* and *IL11RA* and *gp130* expression levels in either preserved (ρ=0.31 and ρ=0.28, respectively) or lesioned (ρ=-0.05 and ρ=-0.14, respectively) tissue (**Supplementary Table 4**).

Table 1 - Gene expression differences in lesioned OA osteochondral explants upon exposure to hrIL11. The genes selected as read-out were cartilage/bone markers (ACAN, COL2A1, SPP1, and RUNX2), genes involved in IL11 pathway (IL11, IL11RA, GP130, and IL6), and genes correlating to IL11 (WNT16, PLAUR, PTGES, and SDC1). Paired GEE was performed to calculate significance.

	Articular cartilage		Subchondral bone	
gene	**FC**	**P-value**	**FC**	**P-value**
ACAN	1,39	9,02E-02	0,94	1,44E-01
COL2A1	1,43	5,46E-01	1,65	3,01E-01
SPP1	**1,87**	**1,59E-02**	1,09	6,43E-01
RUNX2	1,86	4,75E-01	1,05	9,41E-01
IL11	1,00	4,38E-01	1,35	9,84E-02
IL11RA	1,68	1,89E-01	**0,87**	**3,73E-02**
GP130	1,54	8,00E-02	1,00	5,21E-01
WNT16	**0,63**	**6,15E-03**	1,16	1,46E-01
PLAUR	1,31	4,48E-01	1,14	8,60E-01
PTGES	1,89	1,10E-01	**0,79**	**4,12E-02**
SDC1	1,40	6,33E-01	0,87	1,97E-01

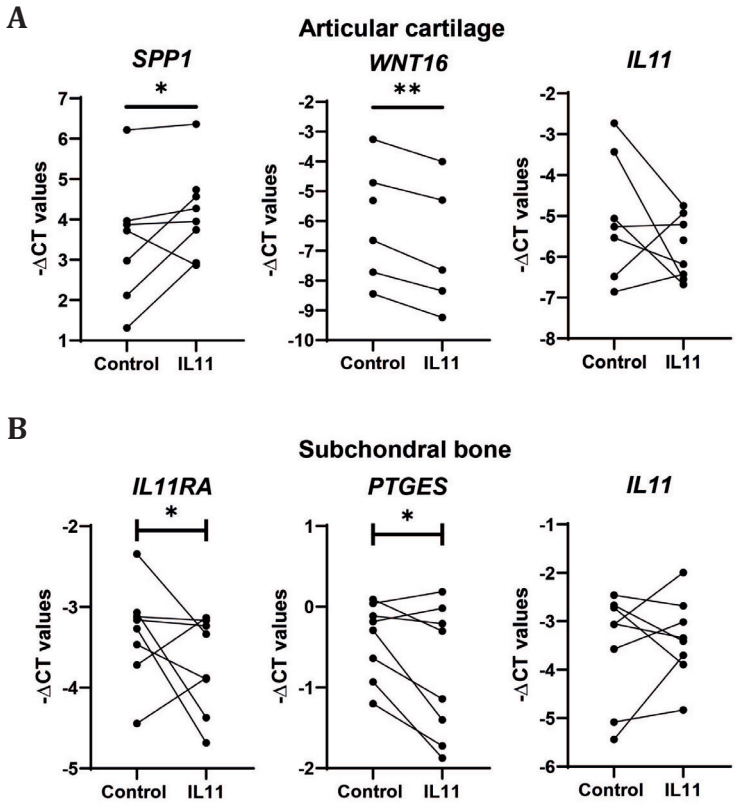


Figure 2 – Gene expression differences in lesioned OA osteochondral explants upon exposure to hrIL11.
(A) Gene expression differences in articular cartilage. (B) Gene expression differences in subchondral bone. Paired GEE was performed to calculate significance. * P<0.05, ** P<0.01, *** P<0.005.

Effects of hrIL11 on lesioned articular cartilage and subchondral bone

Since addition of hrIL11 is previously proposed as therapeutic strategy for OA, we added hrIL11 to the culture medium of macroscopically lesioned osteochondral explants from day 3 onwards and evaluated effects of hrIL11 on cartilage and bone matrix deposition. We used gene expression levels of *ACAN* and *COL2A1* as anabolic cartilage and *SPP1* and *RUNX2* as anabolic bone markers. Moreover, to study *IL11* signaling we used gene expression levels of *IL11, IL11RA,* and *GP130* marking IL11 receptors. Finally, we selected *WNT16, PLAUR, PTGES,* and *SDC1* as genes correlating to *IL11* in joint tissues. To our surprise, the effects of hrIL11 exposure to lesioned osteochondral explants were minimal (**Table 1**). In lesioned cartilage we only observed significant increased expression levels of bone marker *SPP1* (FC=1.87, Pval=1.59x10^{-2}) and decreased expression of *IL11* correlating gene *WNT16* (FC=0.63, Pval= 6,15x10^{-3}) (**Figure 2A**). In lesioned subchondral bone we only observed significant downregulation of *IL11RA* (FC=0.87, Pval=3.73x10^{-2}) and *PTGES* (FC=0.79, Pval=4.12x10^{-2}) (**Figure 2B**). Notably,

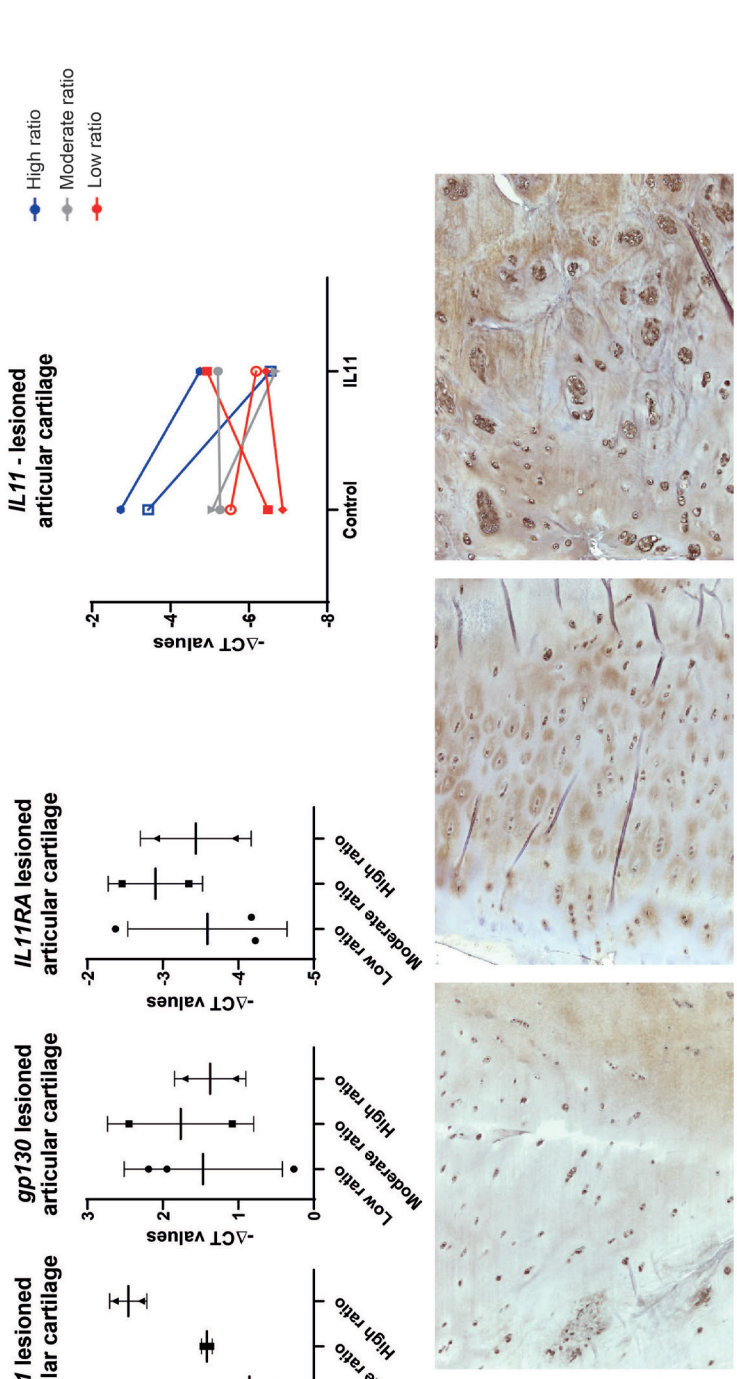


Figure 3 – Expression patterns in lesioned articular cartilage while stratifying for low, moderate, and high IL11:receptors ratio. (A) Expression of IL11, IL11RA, and GP130 in articular cartilage. (B) Response of IL11 gene expression to hrIL11 exposure. (C) Protein expression levels of IL11 in articular cartilage of control osteochondral explants.

intrinsic *IL11* expression did not consistently change upon hrIL11 exposure in lesioned cartilage nor subchondral bone, suggesting lack of signal transduction.

Difference in response to hrIL11 between patients

Exposing lesioned OA osteochondral explants to hrIL11 showed minimal effects as indicated by the low number of significantly differentially expressed genes (**Table 1**). Moreover, we observed considerable donor variation in terms of *IL11* gene expression upon hrIL11 exposure particularly in articular cartilage, suggesting differences in IL11 signaling between patients (**Figure 2A**). Therefore, we explored whether intrinsic gene expression levels of *IL11* and IL11 receptors *IL11RA* and *GP130* could determine the response to hrIL11 in articular cartilage. To this end, we calculated the percentage of *IL11* expression relative to expression of *IL11, IL11RA*, and *GP130* together. Subsequently, we ranked the patients based on this percentage and we stratified the patients in patients with low ratio, i.e. low levels of *IL11* relative to levels of receptors, patients with moderate ratio, and patients with high ratio and plotted *IL11, IL11RA,* and *GP130* expression levels. As shown in **Figure 3A**, the difference in ratio was mainly explained by differences in *IL11* expression levels. Hereto, we evaluated whether these ratios explained the observed variation in response of *IL11* expression levels in articular cartilage upon exposing lesioned osteochondral explants to hrIL11. As shown in **Figure 3B**, patients with high ratios, i.e. high *IL11* expression relative to IL11 receptors, showed consistent downregulation of *IL11* expression upon hrIL11 exposure. Upon performing immunohistochemistry to assess whether intrinsic IL11 protein is being produced in these patients, we observed more staining in cartilage samples of patients with high ratios, confirming that *IL11* is translated into protein (**Figure 3C**). Based on these results, we hypothesize that the downregulation, and thus response, in intrinsic *IL11* observed upon exposure to hrIL11 in patient with high ratio might be due to either less efficient binding of intrinsic IL11 protein binding to its receptor compared to hrIL11 or due to restoration of the balance between trans and classic signaling by addition of hrIL11.

Ratios in RNA-seq dataset of lesioned articular cartilage

Since the sample size of osteochondral explants was relatively low (N=7 donors), we next evaluated whether similar differences in *IL11* gene expression levels were observed in our RNA-seq dataset of articular cartilage. As shown in **Figure 4**, the different ratios were also present in RNA-seq data and the differences between the ratios was mainly described by *IL11* expression levels, similar as we observed in the osteochondral explants.

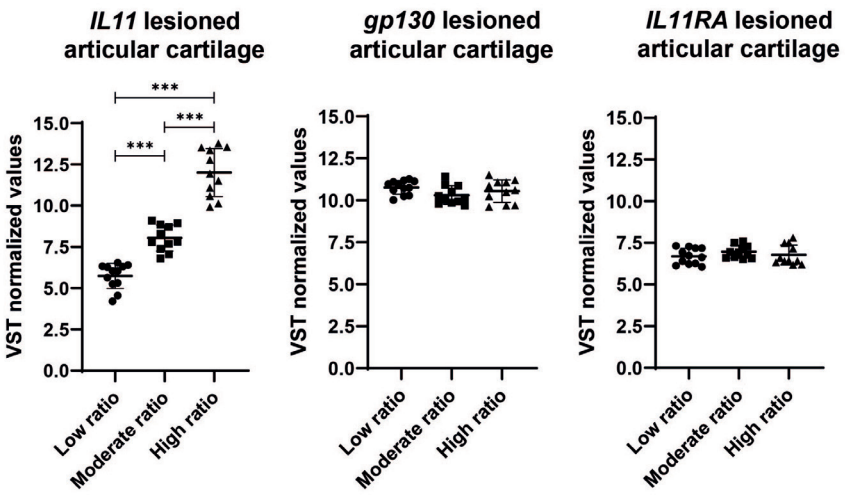


Figure 4 – Expression patterns in lesioned autologous articular cartilage while stratifying for low, moderate, and high IL11:receptors ratio in RNA-seq data.

Discussion

In the current study, we set out to functionally investigate the effect of hrIL11 as potential therapeutic strategy by exposing our previously established biomimetic aged human osteochondral explant model of OA to hrIL11 [15]. Although the effect of hrIL11 exposure on osteochondral explants was generally minimal as reflected by the low number of responsive genes in both tissues (**Table 1**), we did observe significant upregulation of bone marker *SPP1* and downregulation of *WNT16* in articular cartilage, suggesting a more hypertrophic chondrocyte phenotype. Exposure to hrIL11 on subchondral bone only showed significant decreased expression of *IL11RA* and *PTGES,* which potentially is a beneficial response as both genes are known to be involved in osteoclastogenesis and could thus contribute to OA associated bone turnover [13, 21]. Notably, we observed heterogeneity in the response to hrIL11 exposure on articular cartilage between patients, as patients with high *IL11*:receptors ratio in lesioned articular cartilage responded differently compared to patients with low *IL11*:receptors ratio in terms of intrinsic *IL11* gene expression levels. Together, the data presented in this study show that treatment of the whole joint with hrIL11 does not necessarily result in a beneficial response and that heterogeneity between patients should be considered in future studies.

Upon addition of hrIL11 to osteochondral explants, gene expression levels of *SPP1* and *WNT16* were significantly changed in articular cartilage. *WNT16* was previously shown to protect cartilage from degradation and it contributes to cartilage homeostasis by inhibiting canonical WNT signaling [22, 23]. Therefore, the observed significant

downregulation in *WNT16,* together with the increased expression of bone marker *SPP1*, indicates towards an unbeneficial response upon treatment of OA articular cartilage with hrIL11. In subchondral bone of explants exposed to hrIL11, we observed significant downregulation of *PTGES* and *IL11RA*. PGES-1, encoded by *PTGES*, is a protein that converts PGH_2 to PGE_2, a major mediator of inflammation and known to stimulate osteoclastogenesis [24]. Moreover, PGE_2 was shown to enhance bone-resorbing activity of mature osteoclasts [21]. The observed significant decreased expression levels of *IL11RA* suggest a negative feedback loop reducing IL11 signaling. In previous studies it was shown that deletion of IL11 signaling by knocking out IL11RA in mice showed reduced osteoclast differentiation [13]. Together these data suggest that treatment of lesioned OA subchondral bone with hrIL11 results in reduced bone resorption by suppressing osteoclastogenesis and activity of mature osteoclasts. Since it is suggested that excessive subchondral bone remodeling seen with osteoarthritis is due to increased osteoclast activity [25] and multiple studies have shown that osteoclasts activation could also result in cartilage degradation [26-28], the potentially reduced oteoclastogenesis could be a beneficial response for both OA subchondral bone and articular cartilage. The potential unbeneficial response in articular cartilage and beneficial response in subchondral bone together suggest that risk allele rs4252548-T might confer risk to OA via subchondral bone. However, more research is necessary to confirm this including a larger sample size.

We observed variation upon hrIL11 exposure in both articular cartilage and subchondral bone, as indicated by the low number of genes being significantly differentially expressed. One reason for the low number of significantly differentially expressed genes upon hrIL11 exposure could be the relatively low sample size (N=8 patients, N=6-28 osteochondral explants) combined with high donor variation seen with osteochondral explant cultures [15]. Another explanation could be heterogeneity between patients as reflected by a difference in response to hrIL11 due to intrinsic expression levels of *IL11* and its receptors *IL11RA* and *GP130,* as shown in **Figure 3**. Moreover, in our previous study in which we identified two OA molecular endotypes, we observed a different response in terms of *IL11* expression with ongoing OA between these two molecular endotypes (FC=19 and FC=60 between macroscopically preserved and lesioned articular cartilage, respectively) [29]. These molecular endotypes did correspond with the low, moderate, and high ratios reported here, i.e. molecular endotype B was mainly represented among the high ratio patients (**Supplementary Figure 1**). Notably, the frequency of the identified risk SNP is low, which makes it unlikely that there are carriers among the 8 patients that were included, therefore more general effect is expected. Additional research is needed to further elucidate differences between patients based on intrinsic *IL11* expression levels and differences in *IL11* response

to OA pathophysiology. Moreover, identification of non-invasive biomarkers, such as circulating miRNAs, that reflect these intrinsic *IL11* expression level differences might be of added value to enable intrinsic *IL11*-based stratification before starting treatment for example with hrIL11.

On another level, differences on IL11 trans- and classic signaling have been reported and are not captured by the current study [30, 31]. For IL6 it has been suggested that classic signaling, i.e. binding of IL6 to membrane anchored IL6R and gp130, has beneficial effects on cartilage as inhibits metalloproteinases and slightly stimulates proteoglycan production [32, 33]. On the other hand, trans signaling, i.e. binding of IL6 to soluble IL6R and membrane bound gp130, is mostly described being detrimental for cartilage. Moreover, trans signaling is known to be involved in bone resorption, by promoting osteoclastogenesis, and bone formation [32]. Of note is that contradictory results on both classic and trans signaling have also been reported [34]. Similar to IL6 signaling, different effects of classic and trans signaling might occur for IL11 signaling and should be further investigated in future studies.

Next to increasing sample size, culture conditions such as hrIL11 dose, culture period, and culture media composition could still be optimized to obtain more conclusive effects. Of note is that our culture media contains dexamethasone, which is an anti-inflammatory component and could potentially interfere with hrIL11 effects.

Based on genetics it was hypothesized that low levels of IL11 confer risk to OA and administration of hrIL11 protein was suggested as potential treatment for OA. Although previous studies have estimated that drug targets founded by genetic evidence have at least two fold increased success rates [35, 36], we here showed the importance of functionally investigating OA risk genes, as we showed that treating the whole joint with hrIL11 as suggested does not necessarily have a beneficial outcome. Based on our results, treatment of OA articular cartilage with hrIL11 shows unbeneficial effects, while treatment of OA subchondral bone with hrIL11 might be positive for both subchondral bone and articular cartilage. The latter suggest that risk allele rs4252548-T confers risk to OA via subchondral bone.

Declarations

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Disclosures

The authors have no relevant financial or non-financial interests to disclose.

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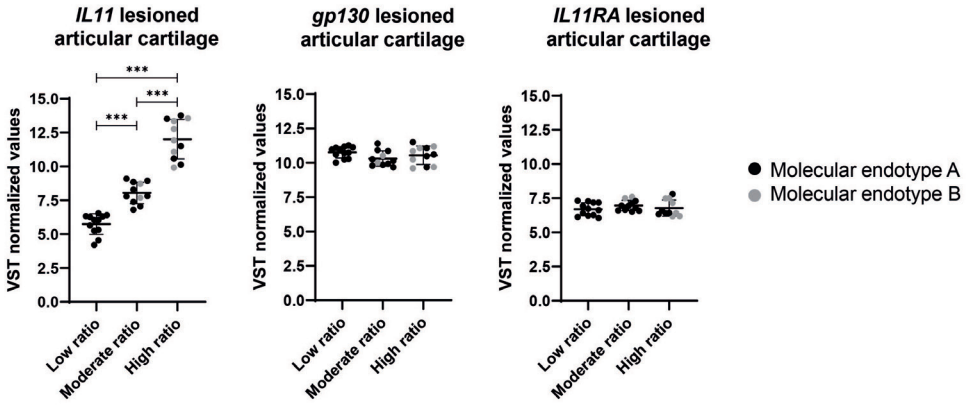
Supplementary files

Supplementary figures



Supplementary Figure 1 - Expression patterns in lesioned autologous articular cartilage while stratifying for low, moderate, and high IL11:receptors ratio in RNA-seq data.
The molecular endotypes A and B are indicated by black and grey dots, respectively.

Supplementary tables

Supplementary Table 1 – Baseline characteristics of material included in the current study.

	RNA-seq data articular cartilage (N=35)	RNA-seq data subchondral bone (N=24)	Osteochondral explants (N=8)
Participants	35	24	8
Age (SD)	68,6 (9,0)	66,2 (8,5)	70,3 (10,9)
Knees (Hips)	28 (7)	18 (6)	8 (0)
Females (Males)	27 (7)	22 (2)	6 (2)

Supplementary Table 2 (partially) – Significant correlations in articular cartilage (|ρ|>0.6 and FDR<0.05).
The top 25 highest absolute correlations between genes expressed in articular cartilage and IL11 in preserved and in lesioned articular cartilage are shown here.

Gene	Ensembl ID	ρ	P-value	FDR	Tissue status
LIF	ENSG00000128342	0.88	3.83E-12	7.68E-08	Lesioned
DRGX	ENSG00000165606	0.81	8.57E-09	6.36E-05	Lesioned
BMP1	ENSG00000168487	0.80	1.08E-08	6.36E-05	Lesioned
WNT7B	ENSG00000188064	0.80	1.27E-08	6.36E-05	Lesioned
S100A2	ENSG00000196754	0.79	2.81E-08	9.60E-05	Lesioned
AP001528.3	ENSG00000280339	-0.79	2.87E-08	9.60E-05	Lesioned
CYTL1	ENSG00000170891	-0.78	6.83E-08	1.96E-04	Lesioned
PLAUR	ENSG00000011422	0.77	7.97E-08	2.00E-04	Lesioned
SPINK1	ENSG00000164266	0.77	9.28E-08	2.01E-04	Lesioned
NGF	ENSG00000134259	0.77	1.00E-07	2.01E-04	Lesioned
KBTBD12	ENSG00000187715	-0.76	1.53E-07	2.80E-04	Lesioned
TPRG1	ENSG00000188001	-0.76	2.01E-07	3.36E-04	Lesioned
SCGB1D2	ENSG00000124935	0.75	2.60E-07	3.40E-04	Lesioned
TNFRSF12A	ENSG00000006327	0.75	2.62E-07	3.40E-04	Lesioned
TNP1	ENSG00000118245	0.75	2.70E-07	3.40E-04	Lesioned
CLCF1	ENSG00000175505	0.75	2.71E-07	3.40E-04	Lesioned
DUSP4	ENSG00000120875	0.75	3.83E-07	4.11E-04	Lesioned
LAMB3	ENSG00000196878	0.75	3.83E-07	4.11E-04	Lesioned
NDRG2	ENSG00000165795	-0.75	3.89E-07	4.11E-04	Lesioned
POMGNT1	ENSG00000085998	0.74	4.60E-07	4.41E-04	Lesioned
ADAMTS14	ENSG00000138316	0.74	4.68E-07	4.41E-04	Lesioned
LINC01711	ENSG00000268941	0.74	4.84E-07	4.41E-04	Lesioned
MPPED1	ENSG00000186732	-0.74	5.80E-07	5.05E-04	Lesioned
PIK3IP1	ENSG00000100100	-0.73	7.63E-07	6.37E-04	Lesioned
RPSAP52	ENSG00000241749	0.73	8.25E-07	6.62E-04	Lesioned
MTHFD2L	ENSG00000163738	-0.78	5.25E-08	7.21E-04	Preserved
COL5A1	ENSG00000130635	0.77	8.30E-08	7.21E-04	Preserved
LINC01711	ENSG00000268941	0.77	1.08E-07	7.21E-04	Preserved
LOXL2	ENSG00000134013	0.76	2.41E-07	1.21E-03	Preserved
ERFE	ENSG00000178752	0.74	4.50E-07	1.80E-03	Preserved
SERPINE1	ENSG00000106366	0.74	6.41E-07	2.14E-03	Preserved
TNC	ENSG00000041982	0.73	1.12E-06	3.22E-03	Preserved

Gene	Ensembl ID	ρ	P-value	FDR	Tissue status
SERPINE2	ENSG00000135919	0.72	1.39E-06	3.49E-03	Preserved
CPM	ENSG00000135678	-0.72	1.57E-06	3.50E-03	Preserved
HERC5	ENSG00000138646	-0.71	2.08E-06	3.91E-03	Preserved
P3H2	ENSG00000090530	0.71	2.14E-06	3.91E-03	Preserved
COL15A1	ENSG00000204291	0.71	2.50E-06	4.17E-03	Preserved
FAM149A	ENSG00000109794	-0.70	4.38E-06	6.45E-03	Preserved
HTRA1	ENSG00000166033	0.70	4.51E-06	6.45E-03	Preserved
IGFBP3	ENSG00000146674	0.69	6.97E-06	9.22E-03	Preserved
NOTUM	ENSG00000185269	0.69	7.36E-06	9.22E-03	Preserved
BMP6	ENSG00000153162	0.68	8.26E-06	9.74E-03	Preserved
GFRA2	ENSG00000168546	0.68	9.16E-06	1.00E-02	Preserved
CRTC3	ENSG00000140577	-0.68	9.52E-06	1.00E-02	Preserved
DNER	ENSG00000187957	0.68	1.12E-05	1.11E-02	Preserved
RPARP-AS1	ENSG00000269609	-0.68	1.16E-05	1.11E-02	Preserved
STK32A	ENSG00000169302	-0.67	1.29E-05	1.17E-02	Preserved
ACADL	ENSG00000115361	-0.67	1.39E-05	1.17E-02	Preserved
DIRAS1	ENSG00000176490	0.67	1.40E-05	1.17E-02	Preserved
SLC39A11	ENSG00000133195	-0.67	1.48E-05	1.17E-02	Preserved

Supplementary Table 3 - gene enrichment of genes correlating to IL11 in articular cartilage.

Supplementary Table 3A - Gene enrichment of genes correlating to IL11 in preserved articular cartilage.

GO-term	Count	%	P-value	FDR	Genes
GO:0005576~extracellular region	28	27.2	3.14E-09	4.48E-07	PLAUR, TNC, DKK3, FSTL3, SERPINE1, AEBP1, LOXL3, TNFSF11, TGFBI, DNAI1, ADM2, COL5A1, CPM, SERPINE2, WNT9A, IGFBP3, BMP6, COL6A3, HTRA1, CLCF1, ERFE, ARSI, NOTUM, NTF3, WNT7B, APOD, LAMB3, COL15A1
GO:0005615~extracellular space	24	23.3	4.48E-08	3.20E-06	TNC, DKK3, FSTL3, SERPINE1, PRKAG2, AEBP1, LOXL3, TNFSF11, TGFBI, COL5A1, LOXL2, CPM, SERPINE2, WNT9A, IGFBP3, BMP6, COL6A3, HTRA1, CLCF1, ERFE, NTF3, WNT7B, APOD, COL15A1
GO:0005788~endoplasmic reticulum lumen	11	10.7	2.51E-06	1.20E-04	PLAUR, TNC, FSTL3, P3H2, COL5A1, IGFBP3, COL6A3, ARSI, NOTUM, WNT7B, COL15A1
GO:0005604~basement membrane	5	4.9	1.36E-03	4.85E-02	TNC, P3H2, TGFBI, COL5A1, LOXL2

Supplementary Table 3B - Gene enrichment of genes correlating to IL11 in lesioned articular cartilage.

GO-term	Count	%	P-value	FDR	Genes
GO:0005615~extracellular space	37	19.5	1.33E-08	3.24E-06	CFH, MYOC, TGFBR3, FSTL3, ASIP, TIMP1, TFPI2, COL7A1, CCL20, FNDC4, IGFBP5, RARRES1, TGFBI, INHBA, PTGIS, SCGB1D2, WNT1, BMP2, LIF, C1QL1, LOXL2, NGF, SEMA7A, HAPLN3, IGFBP4, UCN2, IGFBP1, CPAMD8, FRZB, ANGPTL4, BMP1, CYTL1, CLCF1, GPC5, PAPPA, WNT7B, PDGFA
GO:0005576~extracellular region	40	21.1	4.60E-08	5.61E-06	CFH, PLAUR, TGFBR3, FSTL3, ISM2, TIMP1, TFPI2, COL7A1, CCL20, FNDC4, IGFBP5, NRP2, TGFBI, INHBA, TREM1, WNT1, BMP2, LIF, NGF, CRB1, ADAMTS14, HAPLN3, IGFBP4, UCN2, IGFBP1, ADAMTS1, CPAMD8, FRZB, ANGPTL4, BMP1, ENHO, OSCAR, CLCF1, GPC5, PAPPA, NOTUM, WNT7B, LAMB3, PDGFA, PNP

Supplementary Table 4 - Correlations between IL11 and IL11RA and GP130 expression levels

Tissue	Receptor	ρ	Pval	Padj
Preserved bone	gp130	0.28	1.94E-01	6.83E-01
Preserved bone	IL11RA	0.31	1.56E-01	6.63E-01
Lesioned bone	gp130	-0.14	5.35E-01	7.97E-01
Lesioned bone	IL11RA	-0.05	8.23E-01	9.35E-01
Preserved cartilage	gp130	-0.21	2.34E-01	5.85E-01
Preserved cartilage	IL11RA	0.04	8.06E-01	9.33E-01
Lesioned cartilage	gp130	-0.17	3.41E-01	6.42E-01
Lesioned cartilage	IL11RA	0.06	7.57E-01	9.01E-01

Supplementary Table 5 (partially) - Significant correlations in subchondral bone (|ρ|>0.6 and FDR<0.05).
The top 25 highest absolute correlations between genes expressed in subchondral bone and IL11 in lesioned subchondral bone are shown here.

Gene	Ensembl ID	ρ	Pval	Padj	Tissue status
ELOVL5	ENSG00000012660	-0.88	2.56E-08	4.05E-04	Lesioned
GALK1	ENSG00000108479	0.84	4.20E-07	3.32E-03	Lesioned
CD302	ENSG00000241399	-0.82	1.34E-06	7.06E-03	Lesioned
WNT16	ENSG00000002745	0.81	2.75E-06	1.09E-02	Lesioned
NCOA7	ENSG00000111912	-0.80	4.31E-06	1.36E-02	Lesioned
SDC1	ENSG00000115884	0.79	7.84E-06	1.50E-02	Lesioned
RCHY1	ENSG00000163743	-0.79	8.56E-06	1.50E-02	Lesioned
CUEDC2	ENSG00000107874	0.79	8.56E-06	1.50E-02	Lesioned
CDK2AP1	ENSG00000111328	0.79	8.56E-06	1.50E-02	Lesioned
DICER1	ENSG00000100697	-0.78	1.11E-05	1.66E-02	Lesioned
CHST10	ENSG00000115526	0.78	1.16E-05	1.66E-02	Lesioned
NCOA1	ENSG00000084676	-0.78	1.37E-05	1.80E-02	Lesioned
SORT1	ENSG00000134243	-0.77	1.68E-05	1.84E-02	Lesioned
DNAJC1	ENSG00000136770	0.77	1.68E-05	1.84E-02	Lesioned
SDF4	ENSG00000078808	0.77	1.75E-05	1.84E-02	Lesioned
YIPF2	ENSG00000130733	0.77	1.90E-05	1.87E-02	Lesioned
IFI27L2	ENSG00000119632	0.76	2.22E-05	1.91E-02	Lesioned
KIAA1755	ENSG00000149633	0.76	2.80E-05	1.91E-02	Lesioned
HSDL2	ENSG00000119471	-0.76	2.91E-05	1.91E-02	Lesioned
MME	ENSG00000196549	-0.76	2.91E-05	1.91E-02	Lesioned
AGFG1	ENSG00000173744	-0.75	3.14E-05	1.91E-02	Lesioned
COL7A1	ENSG00000114270	0.75	3.14E-05	1.91E-02	Lesioned
EMC10	ENSG00000161671	0.75	3.14E-05	1.91E-02	Lesioned
PHPT1	ENSG00000054148	0.75	3.26E-05	1.91E-02	Lesioned
YIF1A	ENSG00000174851	0.75	3.26E-05	1.91E-02	Lesioned

Supplementary Table 6 - Gene enrichment of genes correlating to IL11 in lesioned subchondral bone

GO- Term	Count	%	P-value	FDR	Genes
GO:0005783~endoplasmic reticulum	32	16.2	4.28E-06	1.20E-03	ELOVL5, SEC61A1, PDIA5, P4HA2, SDF4, P3H2, CRAT, EEF1D, OGN, MAN1A1, SLC39A7, COL4A3BP, THBS4, P3H1, RRBP1, ERGIC3, OS9, DNAJC1, P3H4, POU2F1, TENM2, DIAPH2, PDE3B, PDIA4, EMC10, ATP1A1, FAM57A, MAP2K1, FKBP2, SULF2, UVRAG, SLC35B4
GO:0005794~Golgi apparatus	30	15.2	5.83E-05	8.16E-03	XYLT2, B4GALT7, ST6GALNAC2, SDF4, P3H2, VTI1B, RABAC1, AP1S1, MAN1A1, SLC39A7, COL4A3BP, CHST10, STK25, B4GALT2, SMO, YIPF2, SORT1, CD36, TLR4, TENM2, PDE3B, PXYLP1, SLC35B2, ATP1A1, MAP2K1, YIF1A, CHST6, ZDHHC17, SLC35B4, B3GNT9