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1 Identification of TGF β signatures in six 2 murine models mimicking different 3 osteoarthritis clinical phenotypes

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7 8 INTRODUCTION

9
10 Although osteoarthritis (OA) is the most prevalent joint disease worldwide, there is still
11 not a single disease-modifying OA drug on the market. The current treatment options usually
12 result in poorly predictable outcomes due to the high interpatient variability in OA clinical
13 and structural features. Indeed, some studies have reported OA phenotype heterogeneity
14 among patients [1-3]. Recently it has been proposed to use advanced techniques to identify
15 combinatorial biomarkers for distinguishing the different OA phenotypes [4], and also to
16 identify patients at higher risk of disease progression, or with different underlying
17 pathophysiologic mechanisms and risk factors [5]. This will help to improve clinical research
18 and to develop targeted treatments and prevention strategies based on a phenotype-guided
19 approach. The advantage of searching for targets based on differences between risk factors
20 is the simplicity then of selecting patients for future personalized medicine.

21 Currently, OA research relies on the use of various animal models (mainly mice and rats,
22 and more rarely large animals) that mimic mechanical, metabolic or inflammatory OA.
23 However, none of these models covers the complexity and heterogeneity of the human

1 disease but different models likely reflect the heterogeneity of human OA. Moreover, it is
2 difficult to compare the results of different experimental studies due to the heterogeneity of
3 animal backgrounds and experimental protocols. Several studies have analysed global gene
4 expression in OA samples using RNA-seq [6-8] and have generated huge amounts of
5 datasets. However, only small subsets of data are validated and large amounts of data are
6 commonly not investigated. To try to tackle some of these limitations, seven French
7 academic laboratories experts in OA animal models formed a Research on OsteoArthritis
8 Disease (ROAD) consortium to centralize many experimental steps and to put in place
9 standard operating procedures (SOP) in order to minimize bias and increase reproducibility.
10 The first objective of the ROAD consortium was to investigate the TGF β pathway in various
11 OA phenotypes. Indeed, recent findings have shown that TGF β is a central player in cartilage
12 homeostasis and OA pathology [9]. However, few data are available on the
13 pathophysiological role of TGF β family members in the different OA phenotypes. Therefore,
14 the consortium analysed the TGF β pathway by transcriptomic analysis in six murine models
15 of knee OA that reproduce the main phenotypes of the human disease: surgical
16 meniscectomy (MNX) to mimic mechanical or post-traumatic OA, hypergravity and MNX
17 (HG-MNX) to mimic overweight-induced mechanical OA, high fat diet and MNX (HF-MNX) to
18 mimic obesity-induced OA, seipin knock-out and MNX (SP-MNX) to mimic metabolic
19 syndrome-induced OA, aging to mimic age-related OA, and collagenase-induced OA (CIOA)
20 to mimic inflammatory OA.

21

22 **MATERIAL AND METHODS**

23

24 **Animal models**

1 Animal models and controls (ten mice/group) were generated using C57BL/6JR6 males that
2 are known to display more severe and reproducible disease [10] and were supplied by the
3 same company (Janvier Labs, France). *Bsc12*^{-/-} mice (SP-MNX and SP-sham controls;
4 C57BL/6J background) were from CEA (Direction des Sciences du Vivant/Genoscope
5 /LABGEM). Six animals per group were calculated to be required to demonstrate significance
6 at the 5% level with a power of 80% using the G*power software but 10 animals were
7 included to have 6 animals with an OA score ≥ 3 at the end of the experiment. MNX was
8 performed in one joint of 10 weeks old mice by the use of partial meniscectomy as described
9 [11, 12] and done by a single trained operator in all laboratories. All animal procedures were
10 approved by the local institutions' animal welfare committees and were performed in
11 accordance with the European guidelines for the care and use of laboratory animals
12 (2010/63/UE). Surgery and euthanasia were performed after anaesthesia with isoflurane
13 gas, and all efforts were made to minimize suffering. Mice were housed in solid bottomed
14 plastic cages in quiet rooms at $22^{\circ} \pm 1^{\circ}\text{C}$, 60% controlled humidity, and 12h/12h light/dark
15 cycle. Animals were used after an adaptation period of 7 days and had free access to tap
16 water and standard pelleted chow (except the HF model). Mice were sacrificed at week 6
17 after OA induction to have a comparable disease time induction although we were aware
18 that OA severity can vary according to the model.

19 • **Joint instability model**

20 MNX was selected as the reference model of joint instability-related OA [11]. Knee joint
21 instability was induced surgically in the right knee by medial partial meniscectomy. Surgery
22 was performed under a binocular magnifier (X15) using a Sharp point microsurgical stab knife.
23 Mice were placed in dorsal position, knee flexed and right foot taped. After skin incision, the
24 medial femoro-tibial ligament was cut, a short incision of the medial side of quadriceps

1 muscle was performed, the knee capsule was cleaved and the patella was sub-luxated
2 laterally. After section of the meniscotibial ligament, the medial meniscus was gently pulled
3 out and $\frac{3}{4}$ of its anterior horn removed. Then, the patella was replaced, the quadriceps
4 muscle and the skin plan sutured. Control animals underwent sham surgery (ligament
5 visualization but not dissection).

6 • **Hypergravity model**

7 Hypergravity mimics the overweight-associated mechanical strain on joints without
8 metabolism dysregulation. In mice with MNX, hypergravity induces large OA lesions that are
9 not observed without surgical induction [13, 14]. MNX was performed on the right knee, and
10 mice were put back in their box for 48 hours. Then, cages were transferred in the gondolas
11 of the centrifuge (COMAT Aérospac, Flourens, France) to maintain a permanent level of
12 hyper gravity [14]. This device has four 1.4m-long arms that hold at their distant end a
13 mobile octagonal gondola (56.2 × 52.0 × 59.2 cm). All gondolas are equipped with an infra-
14 red video surveillance system to monitor the animals' condition and food/water stocks. In
15 the centrifuge, temperature and light conditions were identical to that of control cages. At
16 the start of centrifugation, acceleration was smoothly and gradually increased over a period
17 of 40 sec. The final acceleration was 2 g (29.6 rpm), and animals were kept at 2 g for 6
18 weeks. Animals were provided with enough food and water for 4 weeks. Then, the
19 centrifuge was transiently stopped to allow litter change, animal weighing, and chow and
20 water supply refilling. Control mice with MNX were not exposed to hypergravity.

21 • **Metabolic disorder model**

22 Seipin (SP) knock-out mice are representative of metabolism disorder, which is a feature
23 associated with OA [15]. *Bsc12* deficiency in mice recapitulates the main features of the
24 phenotype of patients with Berardinelli-Seip Congenital Lipodystrophy (BSCL), including

1 almost complete absence of adipose tissue, hyperglycaemia, hyperinsulinemia, and insulin
2 resistance. MNX and Sham surgery were performed in 10 week-old *Bsc12*^{-/-} mice.

3 • **High Fat Diet model**

4 The high fat diet model reproduces the effect of obesity and dysregulated metabolism on OA
5 onset [16]. At the age of six weeks, mice were fed with High Fat Diet (HFD, 60% of calories
6 from fat, Ssniff, EF D12492 (II) mod. Soest, Germany) that was provided ad libitum for 10
7 weeks with the chow changed twice per week. A number of mice 20% higher than the final
8 group size was included to ensure statistical power of the experimentation. MNX was
9 performed at the age of 10 weeks. In absence of surgical induction, mice did not develop
10 spontaneous lesions of OA. The average weekly weight gain ranged from 1 g to 1.5 g, leading
11 to a final weight gain of 73% (mean: 14.6 g) associated with insulin resistance (HOMA-IR:
12 +246%). Considering the large variability generally observed in the final body weight and fat
13 mass, only animals with a final weight gain higher than 70% were analysed.

14 • **Collagenase-induced OA model**

15 The collagenase-induced model (CIOA) is characterized by low grade inflammation of the
16 synovial membrane, leading to OA lesions [10]. A solution of 1 U/5µL type VII collagenase
17 from *Clostridium histolyticum* (Sigma-Aldrich) was prepared in saline solution. At day 0, a
18 small skin incision was performed on top of the patellar tendon. The knee was bended and
19 the collagenase solution (5 µL) was injected in the intra-articular space using a 10 µL syringe
20 (Hamilton) with a 25 gauge needle. On day 2, a second collagenase injection was performed
21 according to the same procedure. Six weeks later, animals were sacrificed. Control animals
22 were injected with saline solution.

23 • **Age-related model**

1 Ageing is the main risk OA factor [17]. C57BL/6JRj mice exhibit mild OA lesions in the knee at
2 the age of 18 to 24 months [18]. Mice were housed with free access to food and water and
3 euthanized at the age of 24 months. Control young mice were kept in the animal facility and
4 euthanized at the age of 16 weeks.

5

6 **Sample preparation for histology and mRNA extraction**

7 After sacrifice, femora and tibiae from 10 knee joints (one joint/mouse) per model were
8 dissected. Skin and muscles were removed and the knee joint was isolated by sectioning the
9 distal extremity of tibiae and proximal part of the femurs. The tibial plateau was isolated
10 from bone at the growth plate interface, by cutting 2-3 mm beneath the cartilage surface.
11 The remaining soft tissues (meniscus, ligaments and synovium) were removed. The tibial
12 plateau was immediately placed in 1mL of TRIzol® Reagent (Life Technologies), snap-frozen
13 in liquid nitrogen, and stored at -80°C till RNA extraction. After isolation, femoral condyles
14 were fixed in 4% paraformaldehyde for 36 hours, and then decalcified in 0.5M EDTA at room
15 temperature for 15 days.

16

17 **Histology**

18 After dehydration in a graded series of alcohol, femoral condyles were embedded in paraffin
19 at 60°C in a tissue processor. On average, 30 serial sagittal sections of 5 µm were cut, and
20 three were chosen at the upper, medium and lower levels every 50 µm from cartilage
21 surface. OA scoring was performed after Safranin O-Fast Green staining, according to the
22 OsteoArthritis Research Society International (OARSI) recommendations [19]. For each
23 animal, the OA score was the highest score obtained at one of the three levels. For each
24 model, all sections were blindly scored by the same three readers.

1

2 **RNA isolation**

3 Tibial plateau samples were prepared in each consortium laboratory and then shipped for
4 centralized RNA extraction that was performed by crushing thawed samples with ceramic
5 beads (Precellys[®] Lysing kit CK28R), using a Precellys[®] 24 tissue homogenizer equipped with
6 the Cryolis[®] cooling unit (Bertin Technologies). Samples underwent three successive lysis
7 cycles at 6500 rpm for 15 sec, spaced by a 5 min lag phase at 4°C, before addition of 200 µL
8 chloroform. After incubation at room temperature for 3 min, the aqueous phase was
9 recovered, 600 µL of 70% ethanol was added, and the solution was transferred to an
10 RNeasy[®] spin column (Qiagen) and the next steps were performed according to the
11 supplier's recommendations. Total RNA was quantified with a Nanodrop[®] instrument and
12 aliquots were frozen at - 80°C. RNA integrity was confirmed with the Agilent[®] RNA 6000 kit
13 on an Agilent Bioanalyzer 2100[®].

14

15 **Transcriptomic analysis**

16 Transcriptomic analysis was performed on Custom TaqMan[®] Array Microfluidic Cards (TAC)
17 that were designed to perform 384 real-time PCR reactions on a ViiA[™] 7 Fast Real-Time PCR
18 System (Applied Biosystems[®]). Custom TAC were designed for the analysis of TGFβ family
19 members (table 1). Reverse transcription was performed using 250 ng of total RNA and the
20 High capacity cDNA Reverse Transcription Kit (Life Technologies). Quantitative PCR was done
21 using cDNA (150 ng) mixed with TaqMan Fast Advanced Master Mix (Life Technologies).
22 After 40 cycles of amplification (95°C for 20 sec and then 95°C for 1 sec and 60°C for 20 sec),
23 data were analysed with the Applied Biosystems[®] Relative Quantification Analysis Module.
24 Amplification curves for each target were individually checked and baselines adjusted, when

1 necessary, to determine the cycle threshold (CT) values. Gene expression was normalized to
2 the mean CT value of four housekeeping genes (*Gusb*, *Hprt*, *Rps9*, *Ppia*) and expressed as
3 relative gene expression using the $2^{-\Delta CT}$ formula or as a fold change expression using the $2^{-\Delta\Delta CT}$
4 $\Delta\Delta CT$ formula.

5

6 **Statistical analysis**

7 Unsupervised two-dimensional hierarchical clustering was generated with mean-centred
8 relative expression values ($2^{-\Delta Ct}$) of 91 genes per sample using XLStat software. Distances
9 between samples were calculated based on the ΔCT values using Pearson's Correlation and
10 average linkage method. The vertical height of the dendrogram shows the Euclidean
11 distances between samples. The two-dimensional scatter plot of Principal Component
12 Analysis (PCA) was performed using XLStat and represents the expression pattern of ($2^{-\Delta Ct}$)
13 sample values of the ten subgroups. When plotting the sample data points, F1 (PCA
14 Component 1 (32.85% variance)) was used as the x-axis and F2 (PCA Component 2 (12.69%
15 variance)) as the y-axis. Data did not assume a Gaussian distribution and were considered
16 unpaired. The statistical analysis was performed between 2 groups for each OA model versus
17 its respective control (MNX vs MNX-sham, CIOA vs CIOA-sham, Aged vs Young, SP-MNX vs
18 MNX, HG-MNX vs MNX, and HF-MNX vs MNX) using the Mann-Whitney test and GraphPad
19 7 (San Diego, CA, USA). Data were expressed as relative expression ($2^{-\Delta Ct}$) or as fold change
20 (fold change of gene expression in one OA sample as compared to its respective control
21 normalized to 1) and represented as median with interquartile range. Differences were
22 considered significant at $p < 0.05$ and $p < 0.01$.

23

24 **RESULTS**

1

2 **Defining the Standard Operating Procedures.** One important feature in the study

3 design was to define the SOP after the harmonization of the experimental protocols (from

4 animal models to transcriptomic analysis) in three consensus meetings of the ROAD

5 consortium. A study workflow was designed (Figure 1). At each step, the analysis technique

6 was performed in a single laboratory by the same operator to avoid experimental bias. The

7 centralized OA scoring of the different models and controls (Figure 2A) showed that OA

8 scores were significantly higher in all models (≥ 3 on a scale of 0 to 5) than in their respective

9 control (score ≤ 2), although variability in control samples was observed (Figure 2B). The

10 concentration of total RNA isolated from tibial plateau samples was not homogeneous

11 among samples, and was significantly higher in the aged, HG-MNX and HF-MNX models than

12 in their controls (young and MNX mice, respectively) (Figure 2C). The RIN score, which

13 estimates RNA quality and integrity, was heterogeneous among samples, with significantly

14 lower scores in samples from the aged and CIOA animals than from their controls (young and

15 CIOA-sham) (Figure 2D). Among all samples, six out of the ten samples per group that met

16 the criteria of selection were analysed by TAC. The mean CT values for the housekeeping

17 genes were significantly higher in the CIOA, HG-MNX and HF-MNX samples than in their

18 controls (Figure 2E). However, the mean CT values for the housekeeping genes were

19 positively correlated with the mean CT values for all genes (Figure 2F). This indicated that

20 the lower expression of housekeeping genes in some samples could be attributed to a lower

21 amount of cDNA loaded in the TAC and not to a differential regulation of the housekeeping

22 genes. We also detected the expression of genes specific for cartilage (type II collagen,

23 aggrecan) or bone (Runx2, Sp7) in all OA models (data not shown), indicating that both

24 tissues were represented in our samples.

1

2 **TGF β signatures according to the experimental OA phenotypes.** Hierarchical
3 clustering and average linkage clustering of the mRNA expression data in the 10 groups of
4 mice (6 OA models and 4 controls) revealed marked differences among groups (Figure 3A).
5 Three main subgroups could be detected: a cluster that included samples from mice with
6 Aging-, HF-MNX-, HG-MNX-related OA; a cluster that included mainly samples from SP-
7 sham, SP-MNX mice; and a cluster of samples from MNX, CIOA, and CIOA-sham. Sham
8 samples did not cluster together, even though most of them are distributed in the last
9 group with the exception of SP-sham, which is closer to SP-MNX. PCA revealed distinct
10 transcriptional profiles among groups that allowed gathering them in three distinct clusters
11 (Figure 3B). One (HG-MNX and HF-MNX) was clearly separated from the other two clusters
12 that included i) CIOA, MNX and young animals, and ii) control groups (sham, SP-sham, CIOA-
13 sham). Conversely, old and SP-MNX animals were set apart from the others.

14 To determine whether a specific gene signature could be associated with the different
15 OA phenotypes, the gene expression profile of each OA group was compared with that of
16 its control: MNX vs MNX-sham, CIOA vs CIOA-sham, Aged vs Young, SP-MNX vs MNX, HG-
17 MNX vs MNX, and HF-MNX vs MNX. The number of significantly deregulated genes was
18 similar in the MNX, SP-MNX, HG-MNX and HF-MNX groups (around 30 genes) (Figure 3C).
19 Conversely, 15 and 47 genes were deregulated in the samples from CIOA and Aged
20 animals, respectively. We identified genes that were common to two or more groups and
21 a gene signature that was specific for each OA model (see Venn diagram in Figure 3D and
22 table 2). Importantly, no gene was deregulated in all six OA models.

23

1 **OA model-specific TGF β signatures.** To further analyse the specific gene signatures,
2 we visualized the genes that were significantly dysregulated (fold change >1.5) in each OA
3 model using Volcano plots. In the MNX model, gene expression profiling revealed that all 30
4 modulated genes were upregulated compared with control (Figure 4A). In the CIOA model,
5 14 of the 15 deregulated genes were significantly upregulated (Figure 4B). Conversely, in the
6 Aging- and SP-MNX-related OA, most genes were downregulated (44/47 and 25/28 genes,
7 respectively) (Figure 4C-D). Finally, in the HG-MNX and HF-MNX models, 70% and 71% of
8 genes were upregulated (Figure 4E-F). Only four genes were differentially regulated between
9 these models: *Smurf2* and *Id2* were upregulated, *Tgfbrap1* and *Lefty* were downregulated
10 only in the HF-MNX model. Altogether, our data revealed that many TGF β family members
11 were deregulated in the different OA subtypes, supporting the key role of the TGF β
12 pathway, whatever the OA risk factor.

13

14 **A *Gdf5*, *Ltbp4*, *Cd36* combinatorial gene signature for OA.** Then, we split the six
15 OA models in two groups. The first group included the OA models related to obesity or fat
16 metabolism (SP-MNX, HG-MNX, and HF-MNX) and/or MNX. The number of shared and
17 specific genes is shown in the Venn diagram (Figure 5A). Most of the modulated genes
18 were common to two or three models, and few genes were specific to each model.
19 However, only *Cd36* was deregulated in all four models. The second group included MNX
20 and the two other most common OA models: inflammation (CIOA) and aging (Figure 5B).
21 Approximately 50% of all deregulated genes were specific to each model and only two
22 genes were deregulated in all three models: *Gdf5* and *Ltbp4*. Analysis of these three genes
23 in all models and their respective controls showed that *Cd36* was significantly upregulated
24 in MNX, SP-MNX, HG-MNX and HF-MNX samples (Figure 5C). *Gdf5* was significantly

1 upregulated in the MNX and CIOA models and significantly downregulated in the Aging
2 model. *Ltbp4* was significantly upregulated in all models, but for the Aging model where it
3 was significantly downregulated. These data suggest that *Cd36* upregulation is a hallmark
4 of trauma-related OA, while the deregulation of *Gdf5* and *Ltbp4* is related to different OA
5 stimuli.

6

7 **DISCUSSION**

8

9 The first objective of the ROAD consortium was to identify specific gene signatures for
10 the main OA clinical phenotypes using six relevant murine models by focusing on the
11 transcriptomic analysis of the TGF β pathway. Although this pathway has been extensively
12 studied in some OA murine models [18, 20, 21], it is quite impossible to compare these
13 results from independent laboratories due to potential biases that may influence gene
14 expression, such as mouse genetic background, age, sex, housing conditions,
15 histopathological scoring subjectivity and inter-investigator variability. Here, we wanted to
16 limit these potential biases by defining SOPs and by centralizing each step of data
17 acquisition and processing, thereby minimizing the risks of failure to identify relevant
18 targets [22-25]. The resulting data allowed the accurate comparative analysis of six models
19 using their respective controls.

20 The main finding of our transcriptomic analysis is the unexpected lack of deregulated
21 genes common to all murine models of OA, although many TGF β family members were
22 deregulated pointing out the critical role played by the TGF β pathway in OA [26]. This
23 might reflect the heterogeneity of responses to the different stimuli leading to similar
24 symptoms, as observed in patients with OA. Differences in the expression pattern

1 between the different models likely relate to the peculiarities and distinct natures of the
2 models. We are also aware that transcriptional regulation of genes may not be reflected
3 at the protein level. Analysis of these differences at the protein level were beyond the
4 scope of the present study but likely warrants further studies. Some genes, such as type II
5 collagen, may be differently regulated depending on the OA model suggesting possible
6 different timings or mechanisms of regulation that warrant further investigation.
7 Heterogeneity may also be emphasized by the individual responses within the same
8 model, thus highlighting the interest of classifying OA phenotypes using relevant
9 biomarkers in the clinic. Heterogeneity might also reflect different stages of OA in the
10 different models but this is unlikely since the OA scores are similar in all models. The
11 absence of a common signature could also be due to the late time point (6 weeks after OA
12 induction and 24 months of age for old mice) chosen for the transcriptomic analysis when
13 the gene expression profile might reflect an adaptive response. However, this time point
14 is relevant for patients in whom OA is generally diagnosed long after disease initiation.

15 Another important finding is the identification of the combinatorial *Gdf5-Cd36-Ltbp4*
16 signature that might discriminate distinct subgroups of OA phenotypes. Indeed, *Cd36* was
17 upregulated in all mice that underwent surgical MNX. CD36 is a membrane-bound protein
18 and the receptor of thrombospondin-1, fatty acid translocase (FAT), platelet glycoprotein
19 4 (PG4) and scavenger receptor class B member 3 (SCARB3). It is expressed in adipocytes
20 and mesenchymal stromal cells isolated from fat tissue, and its expression level correlates
21 with poor differentiation into the chondrogenic lineage [27]. CD36 expression is increased
22 at sites of cartilage injury and co-localizes with developing hypertrophic chondrocytes and
23 the aggrecan NITEGE neo-epitope [28]. In patients with OA, CD36 expression has been
24 significantly associated with the presence of osteophytes, of joint space narrowing, and

1 higher Kellgren-Lawrence score [29]. Moreover, in chondrocytes from patients with OA,
2 expression of thrombospondin 1 (a CD36 ligand) is strongly decreased concomitantly with
3 the increase in CD36 expression [30]. More recently, the anti-inflammatory and analgesic
4 effects of serum albumin in patients with knee OA was related to inhibition of CD36 in
5 synoviocytes, macrophages and chondrocytes [31]. In addition, our study suggests that CD36
6 might be a specific biomarker of post-traumatic OA. CD36 expression should be thoroughly
7 investigated in cartilage and bone samples from patients with different OA phenotypes.

8 We also found that *Gdf5* expression was deregulated in three of the six OA models
9 under study. It was previously shown that a loss-of-function *GDF5* gene mutation results
10 in joint fusions, and a single-nucleotide polymorphism is associated with higher
11 susceptibility to OA [32]. GDF5 deficiency has also been associated with abnormal
12 ligament laxity and subchondral bone remodelling [33]. Several genome-wide association
13 studies (GWAS) have reported the significant association between knee OA and the *GDF5*
14 locus [29, 34-36]. Very recently, a GWAS using the United Kingdom OA Biobank cohort
15 reported that *GDF5* genetic variants were the strongest predictor of knee pain [37]. In the
16 present study, *Gdf5* expression was upregulated in the CIOA and MNX models that are
17 characterized by ligament laxity and pain [10, 38]. Our data strongly suggest that GDF5
18 expression is a biomarker of painful OA phenotypes, as also suggested by genomic studies
19 in humans.

20 Finally, we found that *Ltbp4* was deregulated in all six OA models (Figure 5C), although
21 it was not identified as a deregulated gene common to all models in the statistical analysis
22 (Figure 3D). In the bioinformatic analysis, SP-MNX samples were compared with MNX
23 samples (Figure 4) to investigate the impact of the genetic background on OA. Conversely,
24 in the data presented in Figure 5C, all groups were analysed independently of their

1 control. LTBP4 is a key molecule required for the stability of the TGF β receptor (TGF β R)
2 complex via interaction with TGF β R2, thereby preventing its endocytosis and lysosomal
3 degradation [39]. However, LTBP4 has not been associated with cartilage or OA and unlike
4 its paralogues, LTBP4 is not regulated during chondrogenic differentiation of mesenchymal
5 stromal cells [40]. Like *Gdf5*, *Ltbp4* expression was decreased in old mice and not
6 upregulated as observed in the murine models of induced OA. This suggests that
7 spontaneous aging-related OA might involve different mechanisms.

8 In conclusion, the originality of the present study was to rely on relevant murine models
9 of OA to understand the complexity of OA phenotypes in humans through investigation of
10 the TGF β pathway and based on rigorous SOPs. We did not identify a unique gene signature
11 common to all six OA phenotypes. This highlights the huge heterogeneity of the animal
12 models and the need of caution when extrapolating results from one model to another. But
13 this also highlights that the diversity of the mouse models likely reflects the heterogeneity in
14 human OA. Further studies are needed to validate these potential signatures.

15

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3

4 **AUTHOR CONTRIBUTIONS**

5

6 All authors were involved in revising critically the manuscript and approved the final version.

7 MM: Data analysis, manuscript writing; DN: Experiment design, data analysis, manuscript

8 writing; HKE, DM, MR, EH, XH, DC, MCS, CJa, JYJ, MHLP, PR, JS, CV: Experimental work; FR,

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10

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12

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18

19 **COMPETING INTERESTS**

20

21 The authors declare that they have no competing interests.

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