Transcriptomic analysis of peripheral blood from German Shepherd dogs with osteoarthritis for identification of diagnostic biomarkers

Gabriela Rudd Garces
National Scientific and Technical Research Council
Rosario Vercellini
National University of La Plata
Daniel Osvaldo Arias
National University of La Plata
Pilar Peral García
National University of La Plata
Gisel Padula
National University of La Plata
Guillermo Giovambattista (guillermogiovambattista@gmail.com)
National University of La Plata

Research Article

Keywords: Canis lupus familiaris, RNA-seq, Differentially expressed genes, Joint disease, Precise medicine

Posted Date: April 8th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1513484/v1

Abstract

Canine forms of osteoarthritis (OA) are very similar to those in humans and represent a welfare problem in the dog world population. In this study, we investigated the transcriptomic profile of peripheral blood in German Shepherd dogs with OA in order to identify putative diagnosis biomarkers. The bulk RNA-seq experiment was performed in a cohort of 12 adult dogs, 5 OA-affected and 7 unaffected. Radiographs of the affected dogs revealed signs of progressive OA in hip, elbow and stifle joints. The expression analysis showed 171 differentially expressed genes (DEGs), 113 were upregulated and 58 were downregulated compared to control dogs P (< 0.01). This pool of genes was functional annotated for signaling pathways using PANTHER tools. No overrepresented pathways were found. To gain further insights of the functional role of the DEGs in OA, we set a threshold of log2FoldChange value between -1.5 and 1.5. We ended up with 24 top up- and downregulated transcripts. Prioritization of these DEGs according to their known functional knowledge, revealed five possible candidates for OA biomarkers. The downregulated OSCAR gene encodes the osteoclast associated Ig-like receptor, which is involved in osteoclastogenesis regulation and bone homeostasis. In addition, the upregulated microRNA MIR339-1 and ncRNAs: LOC106559235 (downregulated), LOC102156762 (downregulated) and LOC111096460 (upregulated) are regulatory sequences, stable for gene profiling assessment in blood and related to OA pathogenesis regulation. We suggest OSCAR as the more likely candidate biomarker for OA diagnosis in dogs and, provide evidence of new circulating regulatory sequences differentially expressed in canine OA.

Introduction

Osteoarthritis (OA) is the most common degenerative joint disorder in humans and companion animals (Mele, 2007; Martel-Pelletier *et al.*, 2016; Cimino Brown, 2017). The Lancet Commission on Osteoarthritis reported that more than 500 million people worldwide were affected by this complex disease in 2020 (Hunter *et al.*,2020). The etiology involves metabolic disruption of the articular cartilage, subchondral bone, ligaments, capsule and synovial membrane leading to articular cartilage loss, subchondral bone sclerosis, and inflammation. Progression of these structural alterations leads to joint failure, loss of mobility, pain, and decreased quality of life (Zheng, 2005; Martel-Pelletier *et al.*, 2016). Risk factors associated with OA are heterogenic, for instance, age, gender, obesity, joint biomechanics and genetic background are the most described (Martel-Pelletier *et al.*, 2016; Abramoff and Caldera, 2020). OA commonly appears secondary to hip dysplasia, knee cruciate ligament rupture and avascular necrosis of the femoral (Jacobsen and Sonne-Holm, 2005; Kim, 2012; Simon *et al.*, 2015).

Canine forms of OA are very similar to those in humans and represent a welfare problem in the world dog population. In the UK, an estimated annual period prevalence of 2.5% for appendicular OA has been reported, based on primary-care data. This equates to around 200,000 UK affected dogs annually (Anderson *et al.*, 2018). The most common locations of OA in dogs include the stifle (cranial cruciate ligament rupture and medial patellar luxation), hip (hip dysplasia), elbow (fragmentation of the medial process) and shoulder (osteochondrosis dissecans). While risk factors are similar between owners and pets, inherited defects related to skeletal conformation of certain dog breeds have been associated with

OA developing (Anderson *et al*., 2018). Medium to large breeds such as Border Collie, Bull Mastif, Dogue de Bordeaux, German Pointer, German Shepherd, Golden Retriever, Labrador Retriever, Old English Sheepdog, Rottweiler, Scottish Collie and Springer Spaniel have shown higher odds of OA diagnosis than small and crossbreeds, even in early life (Anderson *et al*., 2018; O'Neill *et al*., 2020).

The diagnosis of OA in human and veterinary medicine is usually made by clinical examination and plain radiography. In some instances, magnetic resonance imaging (MRI) and computed tomography (CT) scans are extremely useful to identify early chondral damage and thus predisposing factors to OA, such as meniscal and cruciate ligament injuries (Abramoff and Caldera, 2020). Different from humans, diagnosis of OA in dogs cannot be assessed based on patient's symptoms and usually by the time owners detect limb movement imbalance and pain in their pets, the disease stage is already advanced (Cimino Brown, 2017; Meeson *et al*., 2019). It has been reported that diagnosis of OA was usually made when dogs were older. In cases with available dates of diagnosis and death, mean proportion of lifespan affected by OA was 11% (Anderson *et al*., 2018). Thus, identifying early articular degradation is the most important challenge in OA research and clinical care.

During the last decade, the use of biomarkers in the diagnosis of OA has gained interest. The rapid development of high-throughput sequencing technologies has enabled the identification of gene expression profiles and networks correlated with OA etiopathology (Mobasheri and Henrotin, 2010; Munjal *et al*., 2019). Interleukins, matrix metalloproteinases, collagen family members, TGF-β pathway-related genes, long noncoding RNA (lncRNA) and microRNAs (miRNA) are some of the most identified in OA gene expression analysis (Reynard and Barter,2020). In dogs, patients and experimentally induced OA have been broadly used to identify OA associated biomarkers. It is difficult to study patients with naturally occurring OA since many factors including breed, age, activity level and severity and duration of the disease are not standardized (de Bakker *et al*., 2017). However, as ethical regulations in animal research have become more important, patients represent the most feasible model (The NC3Rs, https://www.nc3rs.org.uk/). The most used tissues and bio-fluids in OA biomarkers identification are cartilage, synovial fluid, blood and urine (Munjal *et al*., 2019). For instance, in synovial fluid, cytokines, C-reactive protein and matrix metalloproteinase enzymes have been correlated with the early phase of inflammation or tissue destruction. Whereas in cartilage, collagen type II synthesis and degradation products, proteoglycans, hyaluronic acid and cartilage oligomeric matrix protein are associated with long stage joint damage (de Bakker *et al*., 2017). Systemic biomarkers (serum or urine) offer a potential alternative method of quantifying total body burden of OA (Kraus *et al*., 2010), being blood the most suitable due to its easy collection in patients and healthy controls, and role in many of the metabolic pathways, including osteoclastogenesis and bone resorption (Munjal *et al*., 2019).

So far, given the heterogeneous nature of the disease and the lack of sufficiently clinical validation, no single canine OA biomarker stands out as the gold standard (de Bakker *et al*., 2017). Therefore, there is an increasing focus on the development of a panel of biomarkers, which could cover a range of patho-physiological effects, such as cartilage synthesis and degradation, synovitis and inflammation. Combining existing biomarkers may improve their prognostic accuracy and help identify at-risk patients

(Williams, 2009). Thus, identification of biomarkers in peripheral blood, together with the established diagnostic imaging, would enable a more accurate diagnosis of OA, improving canine welfare and avoiding economic losses in veterinary medicine. In this study, we investigated the transcriptomic profile of peripheral blood in German Shepherd patients with OA in order to identify new putative OA biomarkers.

Materials And Methods

Animal cohort and Diagnostic Imaging

Twelve German Shepherd dogs were included in this study. Radiological examination (GBA-Mobilex 150 HF, Argentina and Fujifilm FCR PRIMA II Image Reader / FCR PRIMA Console, Japan) was used to evaluate signs of OA in the dogs. Standard mediolateral radiographs were taken of elbow and stifle joints. In addition, extended ventro dorsal view of pelvis was taken to evaluate coxofemoral joints. Radiographic surveys of multiple joints were performed to record the overall status of the patient (Carrig, 1997). Unspecific signs for OA such as enthesophyte and osteophyte formation, pathologic alteration of subchondral bone (i.e., cyst formation, bone sclerosis and remodeling) and joint spaces varying in thickness were recorded (Allan and Davies, 2018). According to the radiological diagnosis, the animals were grouped in cases (N = 5) and controls (N = 7). The age threshold was set above three years old in control dogs. Body weight, age, sex and neutering status were recorded from each dog by clinical examination.

RNA isolation and cDNA library construction

RNA was isolated from RNAlater stored blood with the RiboPure™-Blood Kit (Ambion, Life Technologies, CA, USA) according to the manufacturer instructions. RNA quality control was assessed with a Bioanalyzer (DEDAE00884, Agilent, Santa Clara, CA, USA). From each sample 1 ug of high quality RNA (RNA integrity number (RIN) > 8) was used for non-stranded, paired-end cDNA library preparation (NEBNext Ultra II RNA Library Prep, Illumina). Multiplexed total cDNA libraries were sequenced on one lane using the NovaSeq 6000 instrument with 2x150 bp paired-end sequencing cycles. The NovaSeq Xp output files with base calls and qualities were converted into FASTQ file format and demultiplexed. Sequence reads were trimmed using fastp program v0.12.5 (Chen *et al.*, 2018). Quality of sequencing data was checked and combined in one FASTQ file using FastQC v0.11.7 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The fastq sequences, processed data and metadata are available in the Gene Expression Omnibus from the NCBI under the accession number GSE191273.

Mapping to reference genome

All reads that passed quality control were mapped to the dog reference genome CanFam3.1 and the NCBI annotation release 105 with HISAT2 aligner v2.1.0 (Kim *et al.*, 2015). Reads were aligned using the default parameters. The alignment of RNA-seq reads from each sample was summarized by the number

of uniquely mapped reads per sample including both singleton and both-ends mapped and number of splice alignments per sample. The HISAT2 output sam format files were transformed into binary format bam files by SAMtools v1.8 (Li *et al.*, 2009). The read abundance was calculated using the featureCounts algorithm (Liao *et al.*, 2014) as part of the Subread package v2.0.1 (http://subread.sourceforge.net).

Differential expression

DESeq2 package (Love and Huber, 2014) was used to read the featureCounts data. Transcripts with zero read in all samples were excluded from further analysis.The count data were subjected to a regularized-logarithm transformation and a principal component analysis (PCA) was performed to visualize the clustering of the case and control groups. Following PCA analysis, we used DESeq2 v1.26.0 to assess differential expression between groups. DESeq2 applies a generalized linear model (GLM) to count data assuming a negative binomial distribution. For each gene, read counts were adjusted to a GLM with design model (~condition) where condition was the factor of interest with two states: OA affected and controls. Transcripts were considered to be differentially expressed with a of P (< 0.01).

Pathway analysis

The enrichment analysis was conducted using the Panther Classification System v16 (Mi *et al.*,2021) and Gene Ontology (GO) database to detect the over-represented biological networks. The gene annotation is given into three groups; biological processes (BP), molecular functions (MF), and cellular components (CC). The gene expression threshold for possible functional role in OA was set for log2FoldChange (< -1.5 and > 1.5) and P (< 0.01) by default.

Results

A cohort of twelve German Shepherd dogs was used in this study, including 5 dogs with OA (1 intact female, 2 neutered females and 2 intact males; dogs between 1 and 8 years of age) and 7 healthy control dogs (1 intact female, 1 neutered female, 3 intact males and 2 neutered males; dogs between 3 and 6 years of age). One OA-affected neutered female was overweight; the rest of the dogs were normal body weight. Radiographic examination demonstrated hip joint involvement in three dogs, elbow and stifle joint involvement in one dog and hip and elbow involvement another one. Radiological signs of dogs with affected hip included joint incongruity such as coxofemoral luxation and subluxation, perichondral osteophyte formation, remodeling of femoral head and neck, subchondral bone sclerosis, flattening of the acetabulum and osteophyte formation on the cranial acetabular margin. Dogs with elbow involvement showed perichondral osteophyte and entesophyte formation, subchondral bone sclerosis and remodeling, increase opacity and blurring over the medial coronoid process and thinning of the joint space (Fig 1B). The dog with stifle OA showed entesophyte formation on the ventral margin of the patella. All of these findings corresponded to severe signs of OA. No records of joint lesions prior to the diagnosis of OA were found in the medical records. However, we cannot formally exclude the prior existence of early-onset hip dysplasia. In contrast, healthy joints showed well defined subchondral bone surfaces and articular margins. The periarticular areas, where ligaments and tendons attach, had a smooth cortical outline. The

joint space appeared as a radiolucent area between adjacent subchondral bone plate surfaces (Fig. 1A). The phenotype data and radiological findings of all dogs are given in Table S1 and File S1.

On average, 20.3 and 36.3 million unique and duplicate reads per sample were collected. A total of 23,284 expressed genes were identified, of which 12,837 were presented in all samples. The output raw matrix is available in File S2.

Figure 2 shows the clustering of samples based on their expression profiles. The PCA did not separate control samples from affected ones. However, assumed that a small set of genes could be differentially expressed between cases and controls, subsequently we applied the GLM model.

Using the GLM model approach we identified 171 DEGs *P* (< 0.01), where 113 genes were upregulated and 58 were downregulated in dogs with OA (Table S2). To get a comprehensive overview of possible functional role in OA, we ranked the DEGs according to log2FoldChange (< -1.5 and > 1.5) and *P* (< 0.01). Twenty-four transcripts were included in the selected threshold (Table 1, Figure 3). Among them, 16 were coding protein genes, three were ncRNAs, two were pseudogenes, one was a microRNA and two (LOC106559672, LOC111094394) were not predicted in the latest reference genome assembly UU_Cfam_GSD_1.0. Prioritization of these transcripts according to their functional knowledge revealed a clear candidate gene with potential relevance for OA. *OSCAR* gene encoding the osteoclast associated Ig-like receptor, is involved in bone and chondrocyte metabolism in OA pathogenesis. Regulatory sequences like microRNA and ncRNA could be also involved in OA process (see discussion).

Table 1 The top 24 up- and downregulated DEGs in peripheral blood of the studied German Shepherd dogs with OA. Transcripts with potential relevance for OA biomarker are highlighted in bold font. Gene annotation corresponds to CanFam3.1 genome assembly and the NCBI annotation release 105.

Genes	BaseMean	log2FoldChange	*P* value	Accession number
LOC106559235	**47.556**	**-1.854**	**2.646^{E-06}**	**NC_006591.3**
LOC106559672	1786.550	3.343	4.684^{E-06}	*
LRRC3C	23.376	2.474	0.0001	+NC_049230.1
LOC475937	112.957	4.556	0.0001	NW_003726568
APOC1	44.068	-3.239	0.0004	NC_006583.3
LOC608055	10.900	1.975	0.0007	NC_006599.3
LOC111096460	**8.380**	**1.934**	**0.001**	**NC_006588.3**
MIR339-1	**13.544**	**1.968**	**0.001**	**NC_006588.3**
LOC611199	10.680	-3.297	0.001	NC_006591.3
LOC111094394	5.713	2.046	0.002	*
TNNI3	15.414	1.845	0.002	NC_006583.3
LOC100687078	18.885	2.141	0.002	NC_006609.3
LOC100683127	26.701	-1.611	0.002	NC_006601.3
EBI3	9.551	-1.504	0.002	NC_006602.3
ADD2	396.485	2.141	0.003	NC_006592.3
HPD	41.968	2.382	0.004	NC_006608.3
FCER1A	133.570	-2.088	0.006	NC_006620.3
CDH24	214.551	1.958	0.006	NC_006590.3
CPA3	544.115	-3.038	0.006	NC_006605.3
OSCAR	**14.586**	**-2.279**	**0.007**	**NC_006583.3**
MPP2	710.079	1.938	0.007	NC_006591.3
FFAR3	10.117	-1.923	0.008	NC_006583.3
LOC102156762	**27.118**	**-2.379**	**0.008**	**NC_006603.3**
LOC608960	11.054	1.501	0.009	NC_006603.3

*This record has been withdrawn by NCBI because the model on which it was based was not predicted in a later annotation. + Accession number corresponding to CanFam3.1 not available in NCBI, the one corresponding to the new dog reference genome assembly UU_Cfam_GSD_1.0 is given.

GO biological process is displayed in Fig 4A. According to the GO categories, the majority of DEGs were classified into cellular and metabolic processes and biological regulation. Within the GO molecular function, DEGs involved in biding and catalytic activities were overrepresented (Fig 4B). The enrichment analysis of the DEGs based on biological pathway is given in Table S3. A total of 44 pathways were enriched; however, neither of them were overrepresented.

Discussion

In this study, we used RNA-seq data from peripheral blood of affected OA and healthy controls German Shepherd dogs to detect the gene expression profile and identify new putative biomarkers for OA diagnosis. Blood samples were used considering that circulating peripheral blood cells are a surrogate tissue containing transcriptional profiles that correlate with OA pathogenesis (Rockett and Burczynski, 2006). Thus, avoiding more invasive sample collections such as synovial fluid or cartilage biopsy and following the National Centre for the replacement, refinement, and reduction of animals in research guidelines. Gene expression studies in blood have indicated networks of co-expressed transcripts with different levels of abundance between OA and healthy controls in humans, horses and rats (Kamm *et al*., 2013; Ramos *et al*., 2014; Korostyński *et al*., 2017; Shi *et al*., 2019). This evidence supports that blood expression profiles may be useful for the evaluation of molecular markers to better diagnose of OA in dogs.

Principal component analysis showed that cases and control did not separate according to the condition. This may be due to only a small dataset among the 12,837 expressed transcripts in all samples is differentially expressed between groups. We identified 171 DEGs, where 113 genes were upregulated and 58 were downregulated in the OA affected German Shepherd dogs. The majority of the DEGs codes for biding and catalytic activities proteins involved in ubiquitous systemic process like immunity, reproduction and metabolism. Among the DEGs, 38 are LOC sequences with undetermined function and orthologs. GO analysis did not show highly enriched pathways, so we assumed that it is more likely to detect isolated potential biomarkers than OA-related pathways in the peripheral blood transcriptome. Subsequently, we applied a threshold with log2FoldChange (< -1.5 and > 1.5) and P (<0.01) to disclose the most up and down regulated transcripts, then ending up with 24 transcripts. We investigated their possible role in OA pathogenesis according to the known functional knowledge

We considered the OSCAR gene a good OA biomarker candidate. It encodes the osteoclast associated Ig-like receptor, is conserved among species and plays an important role in bone homeostasis by osteoclast regulation (Kim *et al*., 2002; Nemeth *et al*., 2011). Osteoclast differentiation is induced by the RANKL cytokine and co-stimulatory signals generated by the transmembrane immunoreceptor tyrosine-based activation motif (ITAM) adaptors, DNAX-activating protein of 12 kDa (DAP12) and Fc receptor common γ (FcRγ) (Koga *et al*., 2004; Mocsai *et al*., 2004). OSCAR associates with FcRγ and provides co-stimulatory signals for osteoclast maturation and activation. RANK-RANKL interaction leads to initial induction of NFATc1, which is amplified through OSCAR/FcRγ-mediated activation of CAMK IV and calcineurin leading to expression of osteoclast-specific proteins. In addition, OSCAR-FcRγ, in association with αvβ3 integrin,

provides signals for cytoskeletal reorganization and thus osteoclast activation. Furthermore, OSCAR is an activating receptor for collagen that co-stimulates osteoclast differentiation during bone development (Barrow *et al*., 2011; Humphrey and Nakamura, 2016; Nedeva *et al*., 2021).

$Oscar^{-/-}$ mice experiment revealed a clear link between this gene and OA development. Oscar deficiency suppresses OA pathogenesis by downregulating the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), and reduced expression of TRAIL in joint tissue inhibits OA cartilage destruction by blocking an apoptotic signal in chondrocytes (Park *et al*., 2020).

Expression levels of OSCAR in human and animal models with degenerative joint diseases have been reported. For instance, enhanced expression of OSCAR was found in peripheral blood monocytes of rheumatoid arthritis patients as compared with healthy controls (Herman *et al*., 2008;). Oscar mRNA and protein levels were markedly elevated during OA pathogenesis in mouse. Similarly, OSCAR was significantly increased in OA damaged regions of human articular cartilage (Park *et al*., 2020). Soluble form of OSCAR has been identified in human serum. In contrast with cartilage and synovial tissue, discrepancy in serum levels of OSCAR was reported. Values in patients with rheumatoid arthritis ranged from decreased, higher, to no significant difference compared to healthy individuals (Herman *et al*., 2008; Zhao *et al*., 2011; Crotti *et al*., 2012; Ndongo-Thiam *et al*., 2014). In our study, we found downregulation of OSCAR in blood of OA affected dogs, which partially agree with previous studies. To the best of our knowledge, there is not available data of OSCAR expression profile in dogs with which we can compare our results. It is unclear whether and how levels of OSCAR in blood and joint tissues correlate.

Furthermore, we identified overexpression of the microRNA (miRNA) MIR339-1 in OA-affected dogs. miRNAs comprise one of the more abundant classes of gene regulatory molecules in multicellular organisms and likely influence the output of many protein-coding genes (Bartel, 2004). Previous studies have shown the key roles of miRNAs in chondrocyte development and cartilage homeostasis by targeting transcription factors or signaling molecules involved in these processes (Miyaki *et al*., 2010; Le *et al*., 2016; Anderson *et al*., 2017). microRNAs have shown an increased expression in human osteoarthritic chondrocytes compared to normal cartilage (Diaz-Prado *et al*., 2012; Balaskas *et al*., 2017). Since miRNAs have been discovered in circulation, their potential role as biomarkers to assess prognosis and/or progression in OA has become attractive. Microarrays profiling together with RT-PCR validation revealed serum miRNA signatures which correlate with risk and disease severity in OA patients (Kong *et al*., 2017; Ntoumou *et al*., 2017). Similar to humans, miRNAs levels in dogs were found sufficiently stable for gene profiling in serum and plasma (Enelund *et al*.,2017). For instance, circulating miR-19b and miR-18a were differentially expressed in dogs with mammary carcinoma (Fish *et al*., 2020). In dogs with appendicular osteosarcoma, circulating miR-214 and miR-126 were successfully assessed as potential biomarkers to predict the outcome of this disease (Heishima *et al*., 2019).

In addition, we detected three ncRNAs highly differentially expressed: downregulated LOC106559235 and LOC102156762 and upregulated LOC111096460. Although, the function of these ncRNAs has not yet been disclosed, in humans lncRNAs have been correlated with OA pathogenesis. A recent study showed

that lncRNA CASC2 was up-regulated in plasma and may participate in OA by inducing cell apoptosis and up-regulating pro-inflammatory factor IL-17 (Huang T *et al*., 2019). Similarly, circulating lncRNA DILC was downregulated in OA patients and showed to be an inhibitor of IL-6, a proinflammatory cytokine in OA (Huang J *et al*., 2019).

Finally, based on the functional knowledge of the analyzed DEGs we suggest OSCAR as a candidate biomarker for OA diagnosis and, provide new evidence of circulating miRNAs and ncRNAs in affected OA dogs. However, since these are preliminary results, we suggest farther validation in larger cohorts of German Shepherd dogs and other OA-susceptible breeds. As well as RT-PCR support of the DGEs candidates. Improving quality of sampling and testing, and measuring large numbers of markers simultaneously in large cohorts would seem likely to identify new clinically applicable biomarkers, which are still much needed in this disease.

Conclusions

In summary, we reported the transcriptomic profile from peripheral blood in OA-affected dogs based on RNA-seq data. We identified high differential expression of OSCAR gene and the regulatory sequences: MIR339-1 (microRNA) and ncRNAs LOC106559235, LOC102156762, and LOC111096460. We propose OSCAR as a candidate biomarker for OA diagnosis in dogs. Our data facilitate panel biomarkers development for diagnosis of OA and thus improved clinical treatments.

Declarations

Acknowledgements The authors are grateful to the dog owners who donated samples and participated in the study. We thank the Interfaculty Bioinformatics Unit of the University of Bern for providing high-performance computing infrastructure. We thank to Jan Henkel and Hernán Morales-Duran for helping with RNA-seq scripts. Analia Arizmendi is acknowledged for making the blood sampling.

Authors' contributions Conceptualization, G.R.G., G.P and G.G; data curation G.R.G; investigation, G.R.G., R.V and G.G; supervision, D.A, G.P and G.G; writing—original draft, G.R.G and G.G; writing—review and editing, G.R.G., R.V., D.A., P.P.G, G.P and G.G.

Funding This study was funded by The National Council for Scientific and Technical Research (CONICET, Grant PUE-2016 N° 22920160100004CO) and The National University of La Plata (UNLP, Grant V247). Gabriela Rudd Garces received a Swiss Government Excellence Scholarship, which enabled her to conduct part of this research

Data availability The transcriptomic data used in this study are available on GEO https://www.ncbi.nlm.nih.gov/geo/info/seq.html.

Statement of Animal Ethics The German Shepherd dogs in this study were privately owned and examined with the consent of the owners. The "Institutional Commission for the Care and Use of Laboratory

Animals" from the Faculty of Exact Sciences, National University of La Plata, Argentina approved the collection of blood samples and radiographs (008-00-17). All dogs were patients of the Small animal hospital of the Veterinary School of the National University of La Plata.

Conflict of interest statement The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Consent to participate Authors have permission to participate.

Consent to publish Authors have permission for publication.

References

1. Abramoff B, Caldera FE (2020) Osteoarthritis: Pathology, Diagnosis, and Treatment Options. Med Clin North Am. Mar;104(2):293-311. https://doi: 10.1016/j.mcna.2019.10.007
2. Allan, G., & Davies, S (2018) Radiographic Signs of Joint Disease in Dogs and Cats. Textbook of Veterinary Diagnostic Radiology, 403–433
3. Anderson BA, Mcalinden A (2017) miR-483 targets SMAD4 to suppress chondrogenic differentiation of humanstem cells. J Orthop Res.35: 2369-77. https://doi: 10.1002/jor.23552
4. Anderson KL, O'Neill DG, Brodbelt DC, Church DB, Meeson RL, Sargan D, Summers JF, Zulch H, Collins LM (2018) Prevalence, duration and risk factors for appendicular osteoarthritis in a UK dog population under primary veterinary care. Sci Rep. Apr 4;8(1):5641. https://doi: 10.1038/s41598-018-23940-z
5. Babraham Bioinformatics (2019) FastQC: A quality control tool for high throughput sequence data. Version 0.11.9. https://www.bioinformatics.babraham.ac.uk/projects/fastqc/. Accessed 22 June 2021. https://doi: 10.12688/f1000research.21142.2
6. Balaskas P, Goljanek-Whysall K, Clegg P, Fang Y, Cremers A, Emans P, Welting T, Peffers M (2017) MicroRNA Profiling in Cartilage Ageing. Int J Genomics.2017:2713725. https://doi: 10.1155/2017/2713725
7. Barrow, A. D. *et al* (2011) OSCAR is a collagen receptor that costimulates osteoclastogenesis in DAP12-deficient humans and mice. J. Clin. Investig. 121, 3505–3516. https://doi: 10.1172/JCI45913
8. Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. Jan 23;116(2):281-97. https://doi: 10.1016/s0092-8674(04)00045-5
9. Carrig CB (1997) Diagnostic imaging of osteoarthritis. Vet Clin North Am Small Anim Pract 27: 777-814. https://doi: 10.1016/s0195-5616(97)50080-5
10. Chen S, Zhou Y, Chen Y, Gu J, 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics. Sep 1;34(17): i884-i890. https://doi: 10.1093/bioinformatics/bty560

11. Cimino Brown D (2017) What can we learn from osteoarthritis pain in companion animals? Clin Exp Rheumatol. Sep-Oct;35 Suppl 107(5):53-58.
12. Crotti, T. N., Dharmapatni, A. A., Alias, E., Zannettino, A. C. W., Smith, M. D., and Haynes, D. R (2012) The immunoreceptor tyrosine-based activation motif (ITAM) -related factors are increased in synovial tissue and vasculature of rheumatoid arthritic joints. Arthritis Res. Ther. 14: R245. https://doi: 10.1186/ar4088
13. de Bakker E, Stroobants V, VanDael F, Ryssen BV, Meyer E (2017) Canine synovial fluid biomarkers for early detection and monitoring of osteoarthritis.Vet Rec. Apr 1;180(13):328-329.https://doi: 10.1136/vr.103982
14. Diaz-Prado S, Cicione C, Muinos-Lopez E *et al* (2012) Characterization of microRNA expression profiles in normal and osteoarthritic human chondrocytes. BMC Musculoskelet Disord.13: 144. https://doi: 10.1186/1471-2474-13-144
15. Enelund L, Nielsen LN, Cirera S (2017) Evaluation of microRNA Stability in Plasma and Serum from Healthy Dogs. Microrna.6(1):42-52. https://doi: 10.2174/2211536606666170113124114
16. Fish EJ, Martinez-Romero EG, DeInnocentes P, Koehler JW, Prasad N, Smith AN, Bird RC (2020) Circulating microRNA as biomarkers of canine mammary carcinoma in dogs. J Vet Intern Med.34(3):1282-1290. https://doi: 10.1111/jvim.15764
17. Humphrey, M. B., and Nakamura, M. C (2016) A comprehensive review of immunoreceptor regulation of osteoclasts. Clin. Rev. Allergy Immunol. 51, 48–58. https://doi: 10.1007/s12016-015-8521-8
18. Heishima K, Meuten T, Yoshida K, Mori T, Thamm DH (2019) Prognostic significance of circulating microRNA-214 and -126 in dogs with appendicular osteosarcoma receiving amputation and chemotherapy. BMC Vet Res. Jan 25;15(1):39. https://doi: 10.1186/s12917-019-1776-1
19. Herman, S., Müller, R. B., Krönke, G., Zwerina, J., Redlich, K., Hueber, A. J., *et al* (2008) Induction of osteoclast-associated receptor, a key osteoclast costimulation molecule, in rheumatoid arthritis. Arthritis Rheum. 58, 3041–3050. https://doi: 10.1002/art.23943
20. Huang J, Liu L, Yang J, Ding J, Xu X (2019) lncRNA DILC is downregulated in osteoarthritis and regulates IL-6 expression in chondrocytes. J Cell Biochem.Sep;120(9):16019-16024. https://doi: 10.1002/jcb.28880
21. Huang T, Wang J, Zhou Y, Zhao Y, Hang D, Cao Y (2019) LncRNA CASC2 is up-regulated in osteoarthritis and participates in the regulation of IL-17 expression and chondrocyte proliferation and apoptosis. Biosci Rep. May 14;39(5): BSR20182454. https://doi: 10.1042/BSR20182454
22. Hunter DJ, March L, Chew M (2020) Osteoarthritis in 2020 and beyond: a Lancet Commission. Lancet. Nov 28;396(10264):1711-1712. https://doi: 10.1016/S0140-6736(20)32230-3
23. Jacobsen, S. & Sonne-Holm, S (2005) Hip dysplasia: a significant risk factor for the development of hip osteoarthritis. A cross-sectional survey. Rheumatology 44, 211–218. https://doi: 10.1093/rheumtology/keh436
24. Kamm JL, Frisbie DD, McIlwraith CW, Orr KE (2013) Gene biomarkers in peripheral white blood cells of horses with experimentally induced osteoarthritis. Am J Vet Res. Jan;74(1):115-21. https://doi:

10.2460/ajvr.74.1.115

25. Kim, D., Langmead, B. & Salzberg, S (2015) HISAT: a fast spliced aligner with low memory requirements. Nat Methods 12, 357–360. https://doi: 10.1038/nmeth.3317
26. Kim HK (2012) Pathophysiology and new strategies for the treatment of Legg-Calvé-Perthes disease. J Bone Joint Surg Am. Apr 4;94(7):659-69. https://doi: 10.2106/JBJS.J.01834
27. Kim, N., Takami, M., Rho, J., Josien, R., and Choi, Y (2002) A novel member of the leukocyte receptor complex regulates osteoclast differentiation. J. Exp. Med. 195, 201–209. https://doi: 10.1084/jem.20011681
28. Koga T, *et al* (2004) Costimulatory signals mediated by the ITAM motif cooperate with RANKL for bone homeostasis. Nature. 428(6984):758–763. https://doi: 10.1038/nature02444
29. Kong R, Gao J, Si Y, Zhao D (2017) Combination of circulating miR-19b-3p, miR-122-5p and miR-486-5p expressions correlates with risk and disease severity of knee osteoarthritis. Am J Transl Res.9: 2852-64.
30. Korostyński M, Małek N, Piechota M, Starowicz K, 2017. Blood Transcriptional Signatures for Disease Progression in a Rat Model of Osteoarthritis. Int J Genomics.;2017: 1746426. https://doi: 10.1155/2017/1746426
31. Kraus, V.B., Kepler, T.B., Stabler, T., Renner, J., Jordan, J (2010) First qualification study of serum biomarkers as indicators of total body burden of osteoarthritis. Public Library of Science One 5, e9739. https://doi: 10.1371/journal.pone.0009739
32. Liao Y, Smyth GK, Shi W (2014) featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics. Apr 1;30(7):923-30. https://doi: 10.1093/bioinformatics/btt656
33. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R (2009) 1000 Genome Project Data Processing Subgroup. The Sequence Alignment/Map format and SAMtools. Bioinformatics. Aug 15;25(16):2078-9. https://doi: 10.1093/bioinformatics/btp352
34. Le LT, Swingler TE, Crowe N *et al* (2016) The microRNA-29 family in cartilage homeostasis and osteoarthritis. J Mol Med (Berl).94: 583-96. https://doi: 10.1007/s00109-015-1374-z
35. Love MI, Huber W, A S (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15(12):550. https://doi: 10.1186/s13059-014-0550-8
36. Martel-Pelletier J, Barr AJ, Cicuttini FM, Conaghan PG, Cooper C, Goldring MB, Goldring SR, Jones G, Teichtahl AJ, Pelletier JP (2016) Osteoarthritis. Nat Rev Dis Primers. Oct 13; 2:16072. https://doi: 10.1038/nrdp.2016.72
37. Meeson RL, Todhunter RJ, Blunn G, Nuki G, Pitsillides AA (2019) Spontaneous dog osteoarthritis - a One Medicine vision. Nat Rev Rheumatol. May;15(5):273-287. https://doi: 10.1038/s41584-019-0202-1
38. Mele, E. (2007) Epidemiology of osteoarthritis. Veterinary Focus 17, 4–10.

39. Mi H, Ebert D, Muruganujan A, Mills C, Albou LP, Mushayamaha T, Thomas PD (2021) PANTHER version 16: a revised family classification, tree-based classification tool, enhancer regions and extensive API. Nucleic Acids Res. Jan 8;49(D1): D394-D403. https://doi: 10.1093/nar/gkaa1106
40. Miyaki S, Sato T, Inoue A *et al* (2010) MicroRNA-140 plays dual roles in both cartilage development and homeostasis. Genes Dev.24: 1173-85. https://doi: 10.1101/gad.1915510
41. Mobasheri A, Henrotin Y (2010) Identification, validation and qualification of biomarkers for osteoarthritis in humans and companion animals: mission for the next decade. Vet J. Aug;185(2):95-7. https://doi: 10.1016/j.tvjl.2010.05.026
42. Mocsai A, *et al* (2004) The immunomodulatory adapter proteins DAP12 and Fc receptor gamma-chain (FcRgamma) regulate development of functional osteoclasts through the Syk tyrosine kinase. Proc Natl Acad Sci U S A. 101(16):6158–6163. https://doi: 10.1073/pnas.0401602101
43. Munjal A, Bapat S, Hubbard D, Hunter M, Kolhe R, Fulzele S (2019) Advances in Molecular biomarker for early diagnosis of Osteoarthritis. Biomol Concepts. Aug 9;10(1):111-119. https://doi: 10.1515/bmc-2019-0014
44. National Centre for the replacement, refinement, and reduction of animals in research (NC3Rs). https://www.nc3rs.org.uk/). Accessed on March 16.
45. Ndongo-Thiam, N., Sallmard, G. D., Kastrup, J., and Miossec, P (2014) Levels of soluble osteoclast-associated receptor (sOSCAR) in rheumatoid arthritis: link to disease severity and cardiovascular risk. Ann. Rheum. Dis. 73, 1276–1277. https://doi: 10.1136/annrheumdis-2013-204886
46. Nedeva IR, Vitale M, Elson A, Hoyland JA, Bella J (2021) Role of OSCAR Signaling in Osteoclastogenesis and Bone Disease. Front Cell Dev Biol. Apr 12;9: 641162. https://doi: 10.3389/fcell.2021.641162
47. Nemeth, K., Schoppet, M., Al-Fakhri, N., Helas, S., Jessberger, R., Hofbauer, L. C., *et al* (2011) The role of osteoclast-associated receptor in osteoimmunology. J. Immunol. https://doi: 10.4049/jimmunol.1002483
48. Ntoumou E, Tzetis M, Bradoudaki M *et al* (2017) Serum microRNA array analysis identifies miR-140-3p, miR-33b-3p and miR-671-3p as potential osteoarthritis biomarkers involved in metabolic processes. Clin Epigenetics. 9: 127. https://doi: 10.1186/s13148-017-0428-1
49. O'Neill DG, Brodbelt DC, Hodge R, Church DB, Meeson RL (2020) Epidemiology and clinical management of elbow joint disease in dogs under primary veterinary care in the UK. Canine Med Genet. Feb 14; 7:1. https://doi: 10.1186/s40575-020-0080-5
50. Park, D. R., Kim, J., Kim, G. M., Lee, H., Kim, M., Hwang, D., *et al* (2020) Osteoclast-associated receptor blockade prevents articular cartilage destruction via chondrocyte apoptosis regulation. Nat. Commun. 11:4343. https://doi: 10.1038/s41467-020-18208-y
51. Ramos YF, Bos SD, Lakenberg N, Böhringer S, den Hollander WJ, Kloppenburg M, Slagboom PE, Meulenbelt I (2014) Genes expressed in blood link osteoarthritis with apoptotic pathways. Ann Rheum Dis. Oct;73(10):1844-53. https://doi: 10.1136/annrheumdis-2013-203405

52. Reynard LN, Barter MJ (2020) Osteoarthritis year in review 2019: genetics, genomics and epigenetics. Osteoarthritis Cartilage. Mar;28(3):275-284. https://doi: 10.1016/j.joca.2019.11.010
53. Rockett, J.C., Burczynski, M.E (2006) Introduction to surrogate tissue analysis. In: Burczynski, M.E., Rockett, J.C.(Eds.), Surrogate Tissue Analysis. Taylor & Francis Group, pp. 3–11.
54. Shi T, Shen X, Gao G (2019) Gene Expression Profiles of Peripheral Blood Monocytes in Osteoarthritis and Analysis of Differentially Expressed Genes. Biomed Res Int. Nov 26;2019: 4291689. https://doi: 10.1155/2019/4291689
55. Simon, D. *et al* (2015) The relationship between anterior cruciate ligament injury and osteoarthritis of the knee. Adv. Orthop.1–11. https://doi: 10.1155/2015/928301
56. Williams, F.M (2009) Biomarkers: in combination they may do better. Arthritis Research and Therapy 11, 130. https://doi: 10.1186/ar2839
57. Zhao, S., Guo, Y.-Y., Ding, N., Yang, L.-L., and Zhang, N (2011) Changes in serum levels of soluble osteoclast-associated receptor in human rheumatoid arthritis. Chin. Med. J. 124, 3058–3060. https://
58. Zheng L, Zhang Z, Sheng P, Mobasheri A (2021) The role of metabolism in chondrocyte dysfunction and the progression of osteoarthritis. Ageing Res Rev. Mar; 66:101249. https://doi: 10.1016/j.arr.2020.101249

Figures

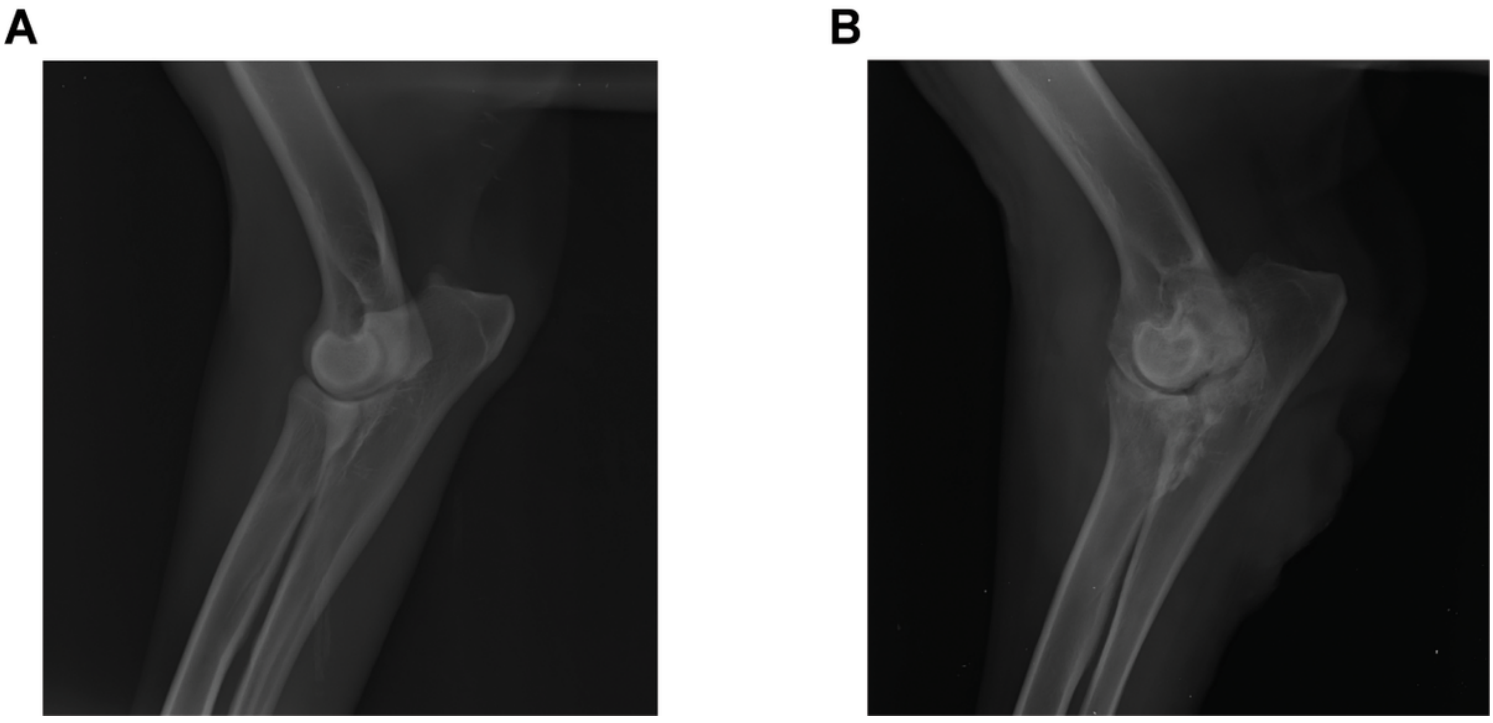

Figure 1

Elbow joint radiographs of two German Shepherd dogs. (A) Mediolateral projection of the normal right elbow joint of a 3 years old male. (B) Mediolateral projection of the right elbow joint of an 8-years old

male with marked OA. Periarticular new bone formation is observed on the coronoid process and on the caudal margin of the humeral throclea. Note the thinning of the joint space and the remodeling of the articular surfaces.

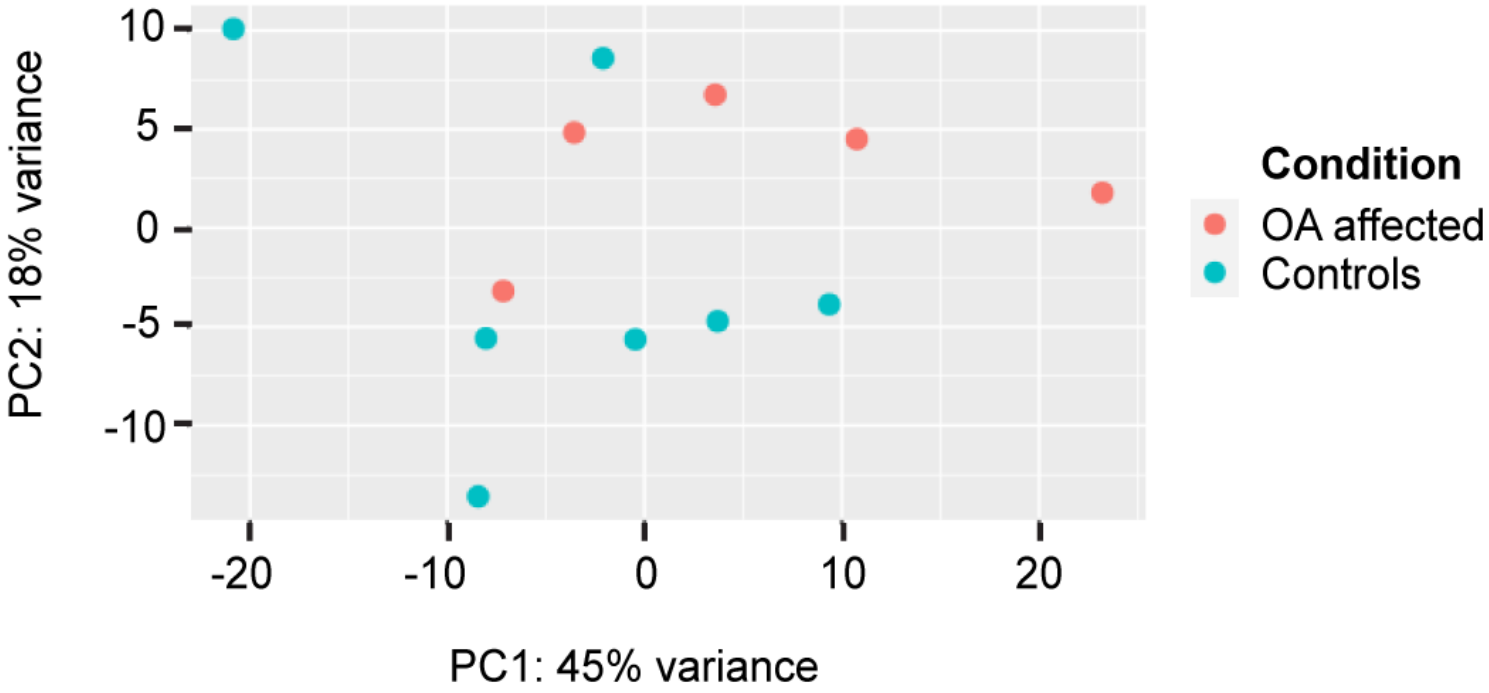

Figure 2

Principal component analysis of the samples in the first two component space. Samples are plotted across the two most variable components (PC1 and PC2); sample clustering is rather based on condition. Notice that the PCA did not separate control samples from affected ones.

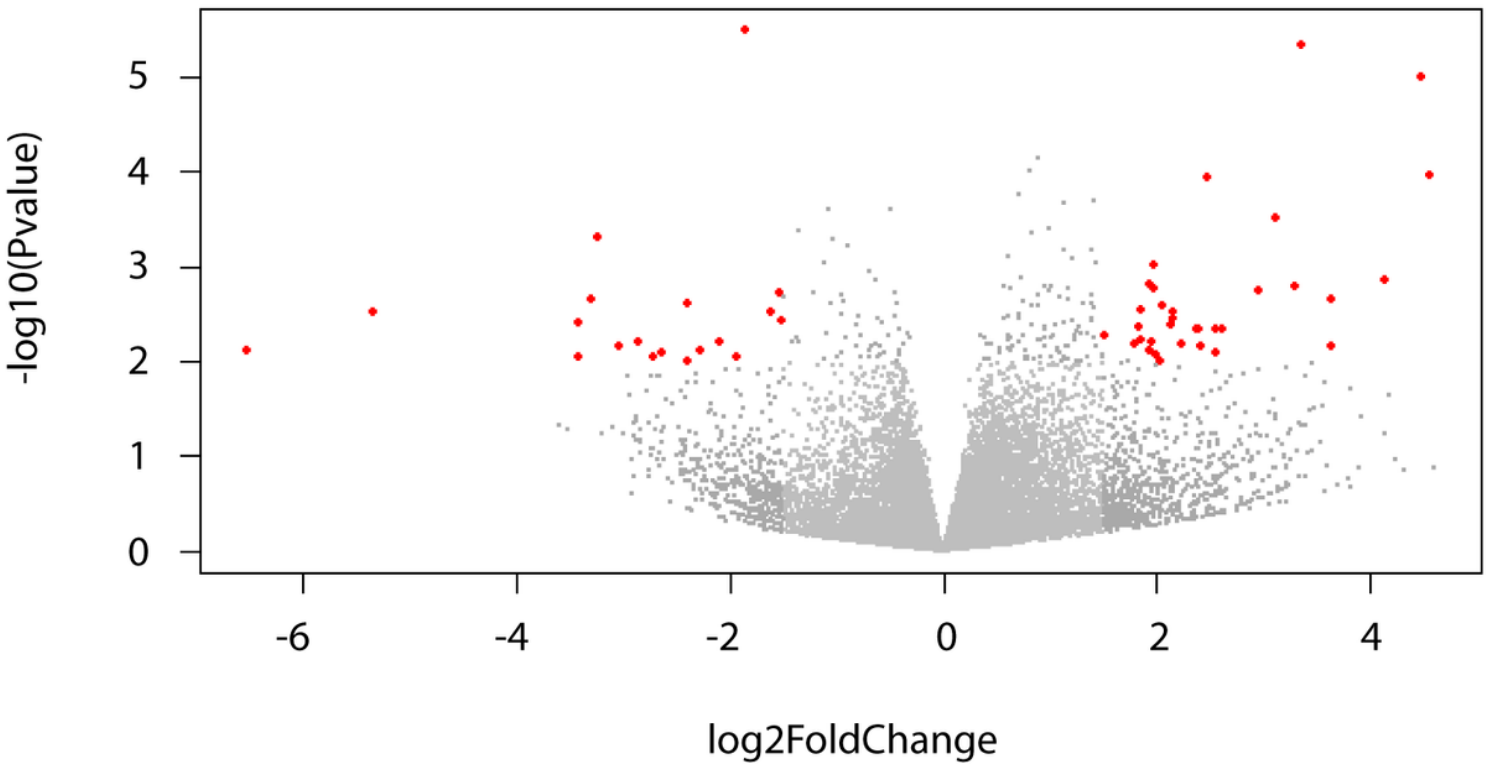

Figure 3

The Volcano plot of the differentially expressed genes (DEGs) between control and cases. The plot visualizes the differences between transcripts in both groups sorted by log2FoldChange and −log10 of *P* value. Red dots represent the top 24 up- and downregulated DEGs with log2FoldChange (< -1.5 and > 1.5) and *P* (< 0.01).

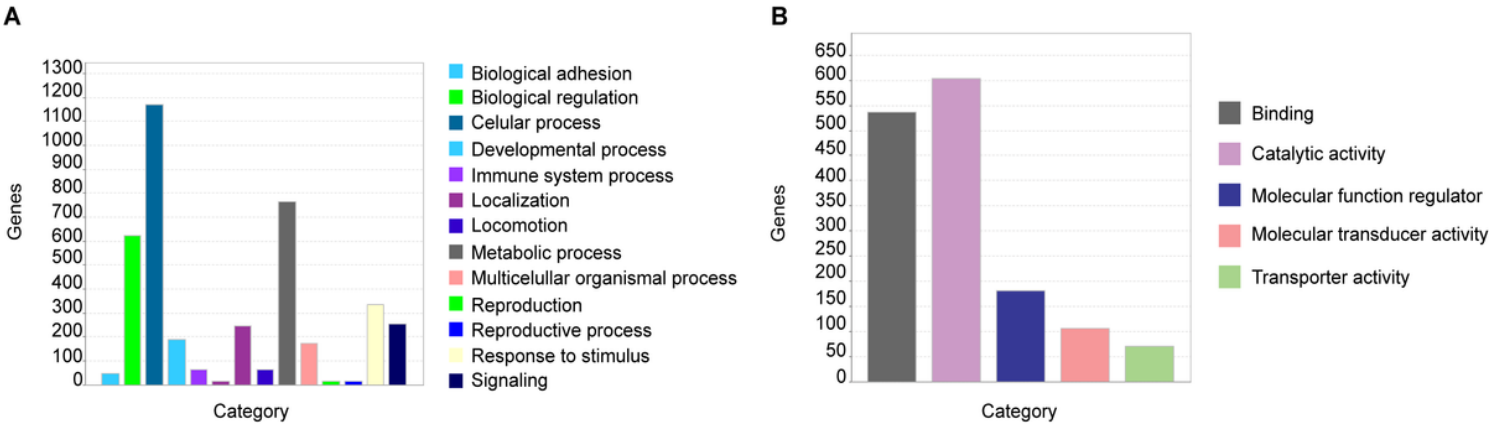

Figure 4

Histograms of the Gene Ontology (GO) classification of differentially expressed genes using the PANTHER Classification System v16. (A) Functional classification based on biological process shows overrepresentation of differentially regulated genes involved in cellular and metabolic processes and

biological regulation. (B) Most of the genes that are differentially regulated within the molecular function category are implicated in biding and catalytic activities.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- FileS1RadiographiesRuddGarceetalVetResCommun.pdf
- FileS2RuddGarceetalVetResCommun.txt
- TableS1.PhenothypedataVetResCommun.xlsx
- TableS2.DifferentialexpresionanalysisVetResCommun.xlsx
- TableS3.EnrichedpathwaysVetResCommun.xlsx