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INTEGRATIVE OMICS PROFILING OF OSTEOARTHRITIS

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Purpose: Osteoarthritis (OA) is a prevalent joint disease that is characterized by the destruction of articular cartilage, although the disease process affects all joint tissues. Understanding the biology of joint homeostasis and the mechanisms of disease will increase our understanding of OA, particularly as it relates to aging. This long-term study aims to integrate genomic, epigenetic, transcriptomic and proteomic data on both normal and OA joint tissues to build a multidimensional molecular profile for OA and aging. The first phase of this project explores the gene expression profiles of articular cartilage from normal and OA human knee joints. In this study, we used RNA-sequencing (RNA-seq) to characterize gene expression profiles, to identify non-coding RNA species and to discover key biological pathways that are dysregulated in OA.

Methods: We extracted total RNA from full-thickness articular cartilage from 20 human donors (10 normal and 10 severe OA) using the Ambion RNAqueous Kit and prepared RNA libraries for sequencing using the NuGen Encore Complete RNA-Seq DR Multiplex System. We used the miRNeasy Mini Kit to purify small RNA from the same donors and prepared the libraries with the Illumina TruSeq small RNA sample prep kit. Sequencing was performed on an Illumina HiSeq 2000 (single-end, 100 bp reads). The reads were trimmed for adapters using CUTADAPT, and the small RNA reads were filtered for lengths ranging from 15-35 nucleotides with a custom Python script. Trimmed and raw data were checked for quality using FASTQC, and the raw fastq files were aligned to the human genome (hg19) using TOPHAT2. Aligned transcripts were assembled with CUFFLINKS2 and differentially expressed (DE) transcripts were determined using CUFFDIFF2 with a q-value less than 0.05, fragment bias correction and upper quartile normalization. Non-coding RNA species were quantified using HTSEQ-COUNT with annotations from NONCODE (Version 4), miRBase (Release 20) and piRNABank for long non-coding RNA (lncRNA), microRNA (miRNA) and piwi-interacting RNA (piRNA), respectively. We conducted a signaling pathway impact analysis using the Bioconductor packages SPIA and Graphite with the KEGG, Reactome, BioCarta and National Cancer Institute/Nature Pathway Interaction Databases (NCI/NPID) to identify potential functional pathways dysregulated in OA.

Results: A total of 862 genes were DE between OA and normal human cartilage (Table 1). One thousand four hundred and thirty miRNAs were expressed in normal cartilage and 1,665 miRNAs were expressed in OA cartilage. Of these expressed miRNAs, 257 were only expressed in OA cartilage, 22 were only expressed in normal cartilage and 37 unique miRNAs were DE (Table 2). Analysis of piRNAs revealed 150 unique piRNAs expressed in OA, 37 of which are DE ($q < 0.05$). A total of 8,642 lncRNAs were expressed in OA articular cartilage and 8 were DE between OA and Normal. Signaling pathway impact analysis revealed 10 significantly perturbed pathways in OA (Table 3)

Conclusions: This integrated Omics profile of OA articular cartilage reveals complex gene expression patterns, potential gene regulatory mechanisms through non-coding RNAs and dysregulated pathways that are involved in the disease. These results provide insights into an integrated, molecular understanding of OA.

Table 1
Gene/Isoform expression summary

Genes		
	Normal	**OA**
Total Genes Expressed	14,531	15,218
Normal Only	24	
OA Only		107
Up-regulated (2-fold or greater difference, $q < 0.05$)		415
Down-regulated (2-fold or greater difference, $q < 0.05$)		447
Known Isoforms		
	Normal	**OA**
Total Known Isoforms Expressed	30,051	31,196
Normal Only	137	
OA Only		296
Up-regulated (2-fold or greater difference, $q < 0.05$)		71
Down-regulated (2-fold or greater difference, $q < 0.05$)		83

Genes and known isoforms expressed in normal and OA articular cartilage.
Expression determined by CuftDiff2, after Benjamini-Hochberg correction. The fold change is the ratio of OA FPKM to normal FPKM.

Table 2
MicroRNA expression levels and log2 (Fold Change) in normal and OA cartilage

Name	**Normal FPKM**	**OA FPKM**	**log2 (Fold Change)**	**q value**
hsa-mir-21	132473	1252710	3.2	0.002
hsa-let-7f-2	10160	91408	3.2	0.011
hsa-mir-142	0	1063	3.0	0.032
hsa-mir-374a	0	2807	3.0	0.009
hsa-mir-1299	0	3965	3.0	0.002
hsa-mir-4318	0	1860	3.0	0.014
hsa-mir-676	0	5108	3.0	0.013
hsa-mir-4510	0	3017	3.0	0.011
hsa-mir-1269b	0	2066	3.0	0.020
hsa-mir-4662a	0	127	3.0	0.027
hsa-mir-146b	163032	1047150	2.7	0.002
hsa-kd-7f-L	3522	20550	2.5	0.044
hsa-mir-100	2612720	14731500	2.5	0.010
hsa-mir-98	9525	48702	2.4	0.011
hsa-mir-155	48783	222402	2.2	0.022
hsa-mir-10b	6266400	26510400	2.1	0.028
hsa-mir-196a-2	18856	78934	2.1	0.016
hsa-mir-195	82217	339757	2.0	0.004
hsa-mir-130a	24770	102014	2.0	0.016
hsa-let-7e	115006	400877	1.8	0.013
hsa-mir-125a	1339370	4645820	1.8	0.023
hsa-mir-26b	389707	1160830	1.6	0.030
hsa-mir-7846	680920	221645	-1.6	0.049
hsa-mir-7108	631835	183699	-1.8	0.023
hsa-mir-4792	1374620	398265	-1.8	0.018
hsa-mir-4734	2789150	796241	-1.8	0.027
hsa-mir-8069	271520	73374	-1.9	0.049
hsa-mir-4767	1438000	384786	-1.9	0.036
hsa-mir-3195	3665880	888282	-2.0	0.004
hsa-mir-4651	1435880	341355	-2.1	0.014
hsa-mir-6814	312896	61907	-2.3	0.013
hsa-mir-1302-1	18718	3102	-2.6	0.014
hsa-mir-614	159705	24408	-2.7	0.017
hsa-mir-4762	59921	8291	-2.9	0.038
hsa-mir-581	1487	0	-3.0	0.002
hsa-mir-4427	2721	0	-3.0	0.015
hsa-mir-4445	4331	0	-3.0	0.002

Table 3
Significantly dysregulated pathways in OA

Rank	Name	pSize	NDK	pNDE	tA	pPERT	pG	pGFdr	pGFWER	Status
1	PI3K Akt signaling pathway	337	21	2.96E-08	-5.30E+01	1.20E-02	8.09E-09	7.93E-07	7.93E-07	Inhibited
2	Circadian rhythm	30	7	2.04E-07	-7.24E+00	5.00E-02	1.98E-07	9.70E-06	1.94E-05	Inhibited
3	HIF-1-alpha transcription factor network	63	10	2.50E-08	1.55E+00	6.68E-01	3.16E-07	3.59E-05	4.49E-05	Activated
4	Validated transcriptional targets of AP1 family members Fra1 and Fra2	37	7	9.46E-07	6.56E+00	2.90E-02	5.05E-07	3.59E-05	7.17E-05	Activated
5	HIF-1 signaling pathway	108	11	5.39E-07	1.13E+01	3.86E-01	3.41E-06	1.11E-04	5.34E-04	Activated
6	C-MYB transcription factor network	77	10	1.79E-07	1.03E+00	8.88E-01	2.65E-06	1.26E-04	3.77E-04	Activated
7	Acute myeloid leukemia	56	7	1.68E-05	2.28E+00	6.82E-01	1.42E-04	2.79E-03	1.39E-02	Activated
8	ECM-receptor interaction	86	6	1.64E-03	-1.34E+01	7.00E-01	1.42F-04	2.79E-03	1.40E-02	Inhibited
9	IL6-mediated signaling events	47	6	6.04E-05	7.06E+00	3.84E-01	2.71E-04	9.61E-03	3.85E-02	Activated
10	HTLV-I infection	260	13	1.28E-04	-8.73E+00	2.93E-01	4.20E-04	1.19E-02	4.74E-02	Inhibited

pSize: number of genes in the pathway; *NDE*: number of DE genes in pathway; *pNDE*: Probability to observe at least NDE genes in the pathway; *tA*: observed total perturbation accumulation in the pathway; *pPERT*: probability to observe a total accumulation more extreme than tA by chance; *pG*: p-value obtained by combinging pNDE and pPERT; *pGFdr*: False discovery rate; *pGFWER:* Bonferroni adjusted global p-values; Status: Direction in which the pathway is perturbed.

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POWERFUL DETECTION OF OSTEOARTHRITIS SUSCEPTIBILITY LOCI BY COMPREHENSIVE EXAMINATION OF CLINICALLY IMPORTANT ENDOPHENOTYPES

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Purpose: Osteoarthritis (OA) is a highly heterogeneous disease characterised by variable clinical features with possibly different genetic aetiologies. Thus far, the few genetic variants that have been robustly associated with broad definitions of OA (n = 13 in Europeans) explain only a small proportion of its heritability. Our aim is to identify novel OA susceptibility variants by examining an expanded set of more homogeneous, radiographically-derived OA endophenotypes relating to joint morphology, specific anatomic pattern of joint involvement, severity and bone response in OA.
Methods: 2,000 knee and 2,000 hip OA cases with radiographs have been genotyped as part of the arcOGEN study on the Illumina Human 610-Quad Beadchip and Illumina HumanOmniExpress Beadchip arrays. Variables relating to joint morphology, specific anatomic pattern of joint involvement, severity and bone responses in OA were extracted from digitised radiographs. Following 1000 Genomes Project-based imputation and stringent quality control >7 million variants were tested for association with each phenotype. Logistic regression was used for binary variables and linear regression was used for continuous variables adjusted for gender. Fixed-effects meta-analysis was used to combine the results from the two genome-wide association studies (GWAS).
Results: Our results indicate that the study of endophenotypes in OA has the potential to dramatically enhance power to detect OA-relevant associations. For example analysis by knee compartment involvement vs population-based controls yielded 25 independent loci for knee OA at $p < 1 \times 10^{-6}$ in contrast to 1 locus detected for knee OA vs controls in the equivalent binary trait GWAS. In hip OA endophenotype analyses several promising signals were identified some of which are found near genes that are very plausible biological candidates for OA. For example in the analysis of atrophic vs hypertrophic hip OA response a strong signal (OR [95% CI] = 2.03 [1.57–2.63], $p = 2.5 \times 10^{-8}$) was detected in the G protein-coupled receptor, GPR98. Polymorphisms in GPR98 and another G protein-coupled receptor (GPR48) have been associated with osteoporotic fracture and low bone mineral density respectively and gpr98 knockout mice have a low bone mass phenotype. Pattern of hip migration (axial/medial vs non-axial/medial migration) shows strong association with variants in LRCH1 (rs754106, p=2.9x10-7) previously suggestively associated with OA and BMP1 (chr8: 22065846, $p = 2.6 \times 10^{-7}$) which induces bone and cartilage development. From the hip morphology studies the strongest signals were detected in the analysis of femoral neck-length-to-width ratio (rs3112954, located in an intron of ZNF385B, $p = 6.5 \times 10^{-8}$ and rs11695150, $p = 8.8 \times 10^{-8}$ located in an intron of PP1R21).
Conclusions: Through a comprehensive examination of radiographically-derived, OA-related phenotypes we have identified several promising signals that point to novel and biologically plausible genes for OA. Our results indicate that, in a heterogeneous disease like OA the study of narrower phenotype definitions closer to the biology of the disease has the potential to dramatically enhance power to detect OA-relevant associations and yield an unprecedented amount of information on OA susceptibility genes. Further replication is required to boost power and validate these associations.

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NEW FUNCTIONAL MICROSATELLITE ASSOCIATED WITH OSTEOARTHRITIS SUSCEPTIBILITY

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Purpose: Considering that two functional microsatellites are associated with OA, in the *BMP5* and *ASPN* genes, and that their effects are poorly represented by neighboring SNPs, we hypothesized that other genetic variants with these characteristics could contribute to OA susceptibility and have escaped detection in GWAS. To test this hypothesis we searched the bibliography identifying six additional functional microsatellites. Two had been already associated with OA in studies with less than 150 patients, in the interleukin (*IL10*) and calcitonin (*CA*) genes, whereas a second microsatellite in *IL10* was studied, but did not show association. Three other functional microsatellites, in the estrogen receptor 2 (*ESR2*), the tyroxine hydroxylase (*TH*) and the macrophage migration inhibitory factor (*MIF*) genes, have never been studied in OA. We analyzed these six microsatellites in a large case-control study.
Methods: The six functional microsatellites were amplified with labeled primers in samples from 3557 patients with OA (1775 of knee OA and 1782 of hip OA) and in 1878 healthy controls of similar age. All patients and controls were of European Caucasian ancestry either from the UK, Greece or Spain. A subset of samples was genotyped twice for quality control. POWERMARKER, CLUMP and GENEPOP were used for analysis. Genotypes of SNPs in the neighborhood of the *BMP5*, *ASPN*, *MIF* and *TH* microsatellites were obtained from the arcOGEN GWAS for the UK samples included in this study. They were used to assess imputation of microsatellite genotypes with the Beagle and IMPUTE algorithms.
Results: Genotypes of the *CA* microsatellite were inconsistent in replication and discarded. Reproducible genotypes were obtained for the other five microsatellites with call rates >0.93. The *MIF* microsatellite showed five alleles (from 4 to 8 repeats) with homogeneous frequency distribution in the three populations. This microsatellite was associated with hip OA with contrasting effects in women (*P* of the Mantel-Haenszel analysis = 0.018) and men ($P_{M\text{-}H} = 0.029$): the 6 repeat allele