### Title

Novel variants for cartilage thickness and hip osteoarthritis: revealing genes implicated in cartilage and bone development




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CpG dinucleotides differs substantially between hips and knees, generic OA related differential methylation was observed in both hips and knees. Furthermore, we show that mere methylation differences do not imply gene regulation and therefore do not per se elucidate epigenetic regulatory properties of OA associated genes. Finally, gene set enrichment revealed that in late-stage disease extracellular matrix remodelling associated genes are still actively regulated epigenetically.

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## NOVEL VARIANTS FOR CARTILAGE THICKNESS AND HIP OSTEOARTHRITIS: REVEALING GENES IMPLICATED IN CARTILAGE AND BONE DEVELOPMENT

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**Purpose**: Hip osteoarthritis (HOA) is one of the most disabling diseases in the elderly. It has a large genetic component estimated between 40-60%. Heritability of hip joint space narrowing is estimated to be around 64%. To date, genome-wide association studies (GWAS) in osteoarthritis (OA) have yielded only few loci, which is partly explained by heterogeneity in the phenotype used to define OA. Therefore, we have chosen to focus on radiographically measured joint-space width (mJSW), a proxy for cartilage thickness and an important underlying intermediate trait for HOA.
**Methods**: We conducted a genome-wide association study of mJSW in a discovery set of 13,013 participants from five different cohorts (Rotterdam Study I and II, TwinsUK, SOF and MrOS) using standardized age, gender and population stratification-adjusted residuals from linear regression. We replicated signals with a P<5*10-6 in 6,168 individuals from 6 independent cohorts: GARP, JoCo, Chingford, GOAL, GOGO and CHECK. We combined results from all studies in a joined meta-analysis using inverse variance weighting (METAL). We also tested for association between mJSW genetic risk score and mJSW/HOA using logistic regression.
**Results**: The discovery analysis (N=13,013) yielded 18 genetic loci with suggestive evidence for association (P<5*10-6). Six of the loci replicated significantly in the replication cohorts (N=6,168). When we combined discovery and replication results, five loci reached genome-wide significance (P<5x10-8), while another 2 loci had suggestive evidence (P<5x10-7) for association. The analysis yielded 2 known HOA loci: 1) SUPT3H and 2) DOT1L, and 5 novel loci. Three of these novel loci were also significantly associated with HOA (P<10-4) in a meta-analysis of 17 studies (n=37,156): 1) TGFA, 2) RUNX2, and 3) FGFR3. FGFR3 and RUNX2 encode proteins known to be involved in bone and cartilage development. TGFA has been shown to be important in experimental OA models in mice; this is the first report linking TGFA to human OA.
We constructed a genetic risk score using the 7 identified mJSW loci and examined association with mJSW and HOA. Within the Rotterdam Study, we found that individuals carrying more than 7 risk alleles (21% of the population) had 11% lower mJSW (P=2*10-11), and a 3 times higher risk for HOA (P=1*10-3) compared to those individuals carrying 0-2 risk alleles.
**Conclusions**: We identified 3 novel loci (TGFA, RUNX2, and FGFR3) and confirmed 2 loci known to be associated with cartilage thickness and HOA. Together, the identified loci for mJSW may be used to risk-stratify individuals for HOA. Our findings provide insight into the genetics of HOA, revealing that cartilage growth and bone developmental pathways may play an important role in the pathology of HOA and serve as targets for future therapies.

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## LOSS OF MITOGEN-INDUCIBLE GENE 6 RESULTS IN DISTURBED CARTILAGE AND JOINT HOMEOSTASIS

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**Introduction**: Osteoarthritis (OA) is a degenerative joint disease which causes destruction of articular cartilage and altered morphology of the supporting bone. Treatment of OA is currently limited to relief of pain symptoms, and does not address the underlying cause. Previous studies in our lab have shown that the cytokine transforming growth factor alpha (TGFα) is upregulated both in a subset of human OA cases and in animals models (Appleton et al., 2007;2010). TGFα binds and activates epidermal growth factor receptor (EGFR) and signalling is attenuated by mitogen-inducible gene 6 (Mig-6) which both blocks EGFR autophosphorylation and induces internalization. Whole body deletion of Mig-6 in mice has been shown to cause multiple phenotypes including the rapid development of joint deformities and cartilage degeneration (Zhang et al, 2005; Jin et al, 2007), however the tissue and cell types involved have not yet been identified.
**Purpose**: To determine the role of Mig-6 and increased EGFR signalling in cartilage degeneration and joint homeostasis through the targeted deletion of Mig-6 in the cartilage of mice.
**Methods**: Mig-6 was selectively deleted in the cartilage of mice using the Cre-Lox system, with floxed Mig-6 (Mig-6fl/fl) and Cre driven by the Col2a1 promoter (Col2-Cre+/-). Animals were kept on a normal diet and activity levels were unregulated. Littermate controls were used in all experiments. MicroCT at 20 and 50 μm/voxel resolutions was used to assess changes in bone density and morphology at 12-36 weeks of age. Alcian blue/alizarin red whole skeletal stains were used to examine early skeletal morphology at 40 days of age (P40). Histological stains including safranin O/fast green, picrosirius red, toluidine blue, tartrate resistant acid phosphatase (TRAP) and immunohistochemistry (IHC) were also used to further determine morphological and molecular changes in cartilage and bone.
**Results**: The overall skeletal morphology and weights of cartilage specific Mig-6 knockout mice (KO, Mig-6fl/fl;Col2-Cre+/-) were comparable to control animals (Mig-6fl/fl;Col2-Cre-/- or Mig-6fl/+;Col2-Cre-/-). However, ectopic cartilage and calcified tissues in the knees of KO animals were observed at early time points (12 weeks) and later in the spine as the animals aged (36 weeks). These tissues formed as highly cellular partially calcified cartilaginous growths at 12 weeks and became progressively more bone-like by 36 weeks. IHC showed increased SOX9 and phosphoEGFR (activated) in both the nodular tissues and articular cartilage in the knees and elbows of KO animals. At 12 weeks the knee articular cartilage of KO animals showed an increase in proteoglycan staining, and a statistically significant increase in thickness and chondrocyte cellularity. However, by 36 weeks proteoglycan staining in KO animals was comparable or worse than controls. Picrosirius red stained knee sections under polarized light also indicated possible disturbance of superficial articular cartilage extracellular matrix structure in KO animals as early as 12 weeks of age. Erosion of bone was observed by both microCT and histology in the knees of KO animals and appeared to be associated with ligament insertion sites. TRAP staining was increased surrounding eroded bone sites, and in the nodular growth and trabeculae of KO animal knees.
**Conclusions**: The negative regulation of EGFR signalling by Mig-6 in Col2a1 expressing tissues is important for the maintenance of joint morphology and homeostasis, and may be involved in regulation of tissue calcification. Mig-6 may be a promising molecule for novel therapeutic interventions for OA and studies evaluating the underlying causes of cartilage degeneration and joint homeostasis.

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## THE MICRORNA-29 FAMILY IN OSTEOARTHRITIS

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**Purpose**: MicroRNAs are short endogenous non-coding RNA molecules, typically 19-25 nucleotides in length, which negatively regulate gene expression. In osteoarthritis (OA), several genes necessary for cartilage homeostasis are aberrantly expressed, with a number of microRNAs implicated in this process. However, our knowledge of the earliest stages of OA, prior to the onset of irreversible changes, remains limited. The purpose of this study was to identify microRNAs involved across the time-course of OA using both a murine model and human cartilage, and to define their function.
**Methods**: Total RNA was purified from whole knee joints taken from mice when underwent destabilisation of the medial meniscus (DMM)