

**Results:** Acetylated-Histone 3 (A-H3) level was increased upon treatment with all 4 HDIs, consistent with effective suppression of HDAC activity. The most effective specific HDI was MS275, which showed similar A-H3 levels to TSA, but exhibited some undesirable effects of pan-HDI. PCI increased A-H3 levels, though not as effective as MS275, but also reduced the negative effects of TSA, such as decreased COX-2 and MMP-3 expression under treatment with IL1 $\beta$ . Also, the additive effect of TSA and IL1 $\beta$  on the secretion of MMP3 was not observed in the case of PCI. Chondrocyte proliferation and survival was unaffected by the tested HDIs after 24 h of culture, with the exception of Droxinostat at concentration over 20  $\mu$ M (decreased proliferation as shown by MTS assay and increased cell death as shown by Live/Dead assay). However, upon longer incubation (48 h), both TSA (200 nM) and Droxinostat reduced chondrocyte proliferation, while MS275, PCI and Tubostatin A increased it.

**Conclusions:** The HDI, PCI, appears to be a promising candidate for reducing inflammatory and catabolic markers in chondrocytes exposed to pro-inflammatory cytokines. Further studies will investigate the effect of increasing PCI concentrations in two *in vitro* chondrocyte-based OA models: (1) IL1 $\beta$  treatment; and (2) exposure to supraphysiological mechanical loading. In addition, MS275 could be a good alternative to pan-HDAC inhibitors, such as TSA, when longer exposure times are needed.

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#### REPLICATION STUDY OF OSTEOARTHRITIS SUSCEPTIBILITY GENES IN HAND OSTEOARTHRITIS IN FINNISH WOMEN

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**Purpose:** Replication studies are needed to confirm the osteoarthritis (OA) susceptibility genes in different study populations and also in different joint sites. We chose to analyze in our hand OA material 21 single nucleotide polymorphisms (SNPs) from 18 genes on which there is suggestive evidence of an association with OA in different joint sites.

**Methods:** Bilateral hand radiographs of 542 occupationally active Finnish female dentists and teachers aged 45–63 years were examined and classified for the presence of OA by using reference images. The genotypes were determined by PCR-based methods. Data regarding finger joint pain and other risk factors were collected by a questionnaire. Associations between the SNPs and hand OA were studied by IBM SPSS statistical package Version 20 using principle component analysis based genetic weighted scores and logistic regression.

**Results:** The analysis resulted in 13 weighted genetic scores. When adjusted for age, occupation, BMI and the other scores, score number 4, including A2BP1 and COX2 SNPs, was associated with hand OA in at least three joints (OR 0.67, 95% CI 0.48–0.94), and score number 8, containing a TGFB1 SNP, with symptomatic DIP OA in at least 2 joints (OR 1.97, 95% CI 1.11–3.49).

**Conclusions:** Our results give support to the earlier findings of A2BP1, COX2 and TBGF1 being OA susceptibility genes, SNPs of which play an important role in the etiology of hand OA in Finnish women.

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#### VARIATION IN OSTEOARTHRITIS BIOMARKER SERUM COMP LEVELS IN MEXICAN AMERICANS IS ASSOCIATED WITH SNPs IN A REGION OF CHROMOSOME 22Q ENCOMPASSING MICAL3, BCL2L13, AND BID

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**Purpose:** Many of the factors that account for variation in OA risk and progression remain elusive, but there is growing consensus that chondrocyte cell death plays a central role in disease pathogenesis. We

performed association analyses in Mexican Americans from the San Antonio Family Study – a group of family-based studies of complex disease genetics – with all SNPs in a region of 22q including the contiguous genes BCL2L13, BID, and MICAL3. This region has been implicated in OA genetics in European populations and harbors two candidate genes, BCL2L13 and BID, both of which are pro-apoptotic members of the BCL-2 family of apoptosis proteins.

**Methods:** OA serum biomarkers such as COMP are valuable quantitative endophenotypes for susceptibility gene localization and identification. They can reflect a broad range of disease pathogenesis, and being closer to the direct action of genes, they may lead to more rapid discovery of genes causally involved in OA risk. We measured COMP levels in stored serum samples using ELISA (BioVendor R&D) in 840 individuals. We tested for association between 428 SNPs in the region 500kb upstream, downstream, and including MICAL3, BCL2L13, and BID and quantitative variation in circulating COMP concentration using the software package SOLAR.

**Results:** The effective number of SNPs in the region, considering LD was 191, requiring  $p=0.00027$  for statistical significance. Three SNPs, rs1080199, rs2535707, and rs5992088 were significantly associated with variation in serum COMP levels. Each occurs in BCL2L13, and the best SNP (rs1081099) explains ~2.6% of the variance in COMP levels. The average LD among the 3 SNPs is 0.94.

**Conclusion:** Our findings are consistent with those of others who have reported associations of genes located in chromosome 22q with OA risk and progression. Given that chondrocyte apoptosis is a feature of OA pathogenesis and COMP is a potent suppressor of apoptosis in primary chondrocytes, our result is particularly interesting in light of the presence of two pro-apoptotic genes in that region: BCL2L13 and BID. Members of the BCL-2 family of proteins, they both function in the mitochondrial apoptosis pathway, which is altered in OA. Chondrocyte mitochondrial dysfunction has been observed in OA joints; consequently, variants in BCL2L13, BID or even nearby MICAL3 could, by extension, affect OA susceptibility and progression. It is encouraging that the results of our study of data from Mexican American families provides additional evidence that this region of chromosome 22q is likely to harbor genes important to OA risk and also implicates biologically promising candidate genes.

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#### CORRELATION BETWEEN CLINICAL AND RADIOGRAPHIC CLASSIFICATION OF OSTEOARTHRITIS AND SNPs LINKED TO OSTEOARTHRITIS SUSCEPTIBILITY

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**Purpose:** Osteoarthritis (OA) classification is based mainly on clinical and radiographic evaluations. Clinical examination can use specific scores for each joint, such as the Knee Society Score (KSS), while the Kellgren and Lawrence (KL) scale is commonly used for x-ray evaluation. Today, proteogenomic studies have taken an increasingly significant role in the etiology of OA. Indeed, several authors have shown that genetic and environmental factors could play a relevant role in OA not yet classified. Moreover functional polymorphisms in frzb, matn3, aspn, pthr2, gdf5 and dvwa genes increase susceptibility to OA development. The aim of this study was to correlate clinical and radiographic grading of OA with molecular data. This study is based on the analysis of genetic polymorphisms linked to OA susceptibility and proteogenomic analysis in OA patients.

**Methods:** We enrolled 61 patients with primary OA, aged 54–86 years, candidate for knee surgery with arthroscopy or arthroplasty. Clinical evaluation was performed using the Knee Society Score: Knee Score (KS) and Function Score (FS). The radiographic evaluation was performed with the KL scale on anteroposterior and lateral x-ray views of the knee. The classification is synthesized into 3 groups: A (KL grade 1 and 2), B (grade 3), C (grade 4).

Human Gene Mutation Database and dbSNP Short Genetic Variations database were used to analyze gene regions containing the selected SNPs. Patient genotypes were obtained by sequencing analysis approaches. Data correlations were analyzed using specific statistical algorithms as follows: association between clinical (KS, FS, BMI, Age) and radiographic data; association between genotypes and radiographic group of OA severity (A, B, C), association between genotypes, radiographic severity of OA and clinical data.

**Results:** Within the 61 patients enrolled in this project, 37 were female and 24 male. The A group consisted of 20 patients, the KS was poor in 11 cases and fair in 9 cases, the FS score was measured from 10 to 90 points. The B group consisted of 21 patients, the KS was poor in 19 cases and fair in 3 cases, the FS score was measured from 10 to 70 points. The C group consisted of 20 patients, the KS was poor in all 20 cases, the FS score was measured from 5 to 50 points. Clinical and radiographic data were associated with SNPs genotype panel obtained for each patient.

**Conclusions:** Correlations between clinical and radiographic data support the results reported in literature. Association between genotypes and radiographic group of OA severity was statistically significant ( $p\text{-value} < 0.05$ ) not for all OA susceptibility polymorphisms at present known. Moreover, association between genotypes, radiographic severity of OA and clinical data, was statistically not significant ( $p\text{-value} > 0.05$ ). This study shows that genetic analysis could have a significant role in the etiopathogenesis of the OA disease and specific SNPs could be potentially used as markers of disease progression in association with clinical and radiological analysis.

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#### GWAS OF SELF-REPORTED OSTEOARTHRITIS IN MEXICAN AMERICANS FROM THE SAN ANTONIO FAMILY STUDY

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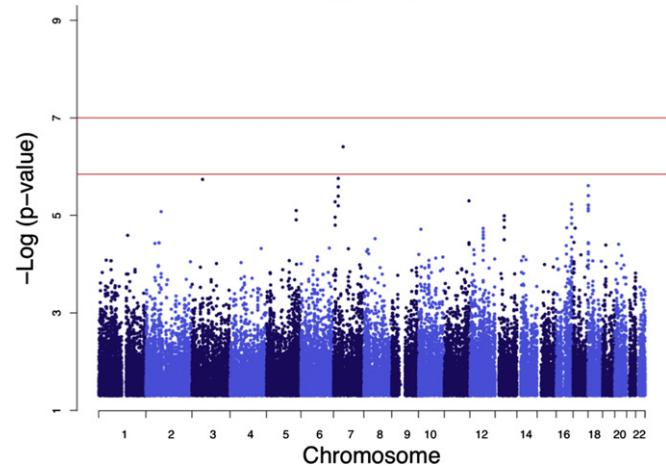
**Purpose:** The identification of genetic factors involved in disease progression and etiology could lead to rapid and significant progress in OA research. The purpose of our study was to identify genomic regions potentially associated with OA disease risk and/or progression through a genome-wide association study (GWAS) of self-reported arthritis in a family-based cohort study with more power to identify rare genetic variants than commonly used case control studies.

**Methods:** We performed this study in 947 adults from the San Antonio Family Study – a group of family-based studies of complex disease genetics. Self-reported OA status was obtained during a general health status interview. Mean serum levels of HA and COMP (obtained via ELISA from stored serum samples) were compared between groups to evaluate the quality of the self-reported status data. OA heritability was assessed using a variance decomposition approach. All analyses were conducted in the software package SOLAR.

**Results:** Serum levels of COMP and HA were strikingly higher in those individuals with self-reported OA ( $p=4.4 \times 10^{-11}$  and  $p=2.6 \times 10^{-8}$  respectively) and this trait shows significant additive genetic heritability ( $h^2=0.26$ ,  $p=0.01$ ). Our strongest evidence for association falls short of genome-wide significance (see Fig.) but exceeds the suggestive threshold. This SNP, rs11506039 ( $p=3.9 \times 10^{-7}$ ), on Chr. 7 is located in an intron of IKZF1. Two other SNPs fall just below the genome-wide suggestive threshold. Also on Chr. 7 is rs10215435 ( $1.7 \times 10^{-6}$ ), located in an intron of OSBPL3; and rs9821479 ( $1.8 \times 10^{-6}$ ) on Chr. 3 may be of interest because of known OA relevance. This intergenic SNP is near WNT5A which has been implicated in rheumatoid arthritis (RA), bone-related signaling and inflammatory response. Also near this SNP is the gene ERC2, which has been associated with bone mass. Two additional associations, also reported by others, may be worthy of note. One, rs1595005 ( $2.5 \times 10^{-6}$ ), is an intronic variant in LINC00470, a chromosome 18p11.32 gene associated with exercise vs. sedentary behavior. The second is rs17192152 ( $5.9 \times 10^{-6}$ ), an intronic variant in CDH13, a gene on 16q23.3 associated with RA.

**Conclusions:** In addition to implicating both recognized candidate loci and novel genes, the results of this GWAS suggest that self-reported OA may be informative for genetic studies of this disorder.

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#### THE LONGITUDINAL RELATIONSHIP BETWEEN ALIGNMENT AND JOINT SPACE WIDTH: DATA FROM THE OSTEOARTHRITIS INITIATIVE (OAI)

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**Purpose:** Malalignment is a potent predictor of knee osteoarthritis (OA) progression, mediates the effects of other risk factors and may be related to incident disease. To date, most studies have used a one-time measurement of alignment, and the longitudinal relationship between alignment, joint space width and other factors is not clear. Using a previously validated method of femoro-tibial alignment (FTA) we measured alignment in a large longitudinal series of OAI subjects in order to describe the longitudinal relationship between FTA and medial joint space width (JSW), both measured annually for 5 years.

**Methods:** We studied OAI subjects who had location specific medial JSW measurements ( $x=0.25$ ) available for five time points (baseline, 12, 24, 36, 48 months), and where an HKA measurement was available. PA radiographs were acquired in standardized fashion with a fixed flexion protocol (<http://www.oai.ucsf.edu/>). FTA was then measured from the same radiographs using a previously published validated method (Duryea et al, 2012). Data for age, gender, race, body mass and Kellgren-Lawrence (KL) grade were collected.

**Analysis:** Descriptive statistics were generated for baseline data. The average change in JSW and FTA from baseline to each time point was calculated. Multivariate models for repeated measures were used to test the independent association between the change in JSW over time and alignment, while adjusting for gender, age, BMI and race. Since alignment likely affects incidence and progression differently, we divided the cohort into knees with baseline KL grades of 0 or 1 and baseline KL grades of 2 or higher. We further separated the cohort into varus and valgus alignment based on the HKA measurement, since medial JSW is likely to vary according to direction of alignment progression. Within each sub-group (KL0-1/ KL2+, varus/valgus) we plotted average annual change in FTA and JSW in those whose disease progressed (increased at least one KL grade) versus those whose KL grade remained stable across all time points.

**Results:** We studied 1172 knees from 787 subjects. At baseline, the sample had an average age of 61.6 (SD 9.1, range: 45–79) and an average BMI of 29.1 (SD 4.9), was 59% female and 85% white. 775 knees were from the Progression cohort, 379 from the Incidence cohort and 18 from the control cohort. There were 350 knees with baseline KL0 or 1 and 822 baseline 2+, while 371 had valgus angulation on HKA and 801 varus. All multivariate models showed a strong relationship between FTA and JSW ( $p < 0.001$ ). Plots of average annual change in FTA and JSW in the various subgroups are presented in Figures 1–4. In progressors, there