genotype ($p < 0.05$). In radiological features, no changes was observed in vitamin D group whereas in placebo group decreased Medial-JSW and increased Osteophyte was observed in Tt and tt genotype in comparion to TT genotype of TaqI polymorphism. These changes were of borderline significance. No similar effect was observed for ApaI polymorphism.
Conclusions: VDR gene polymorphism (TaqI) influences the clinico-radiological response to vitamin D supplementation in Osteoarthritis knee with insufficient 25-OHD levels. If these results are confirmed by the larger studies, this would justify tailoring vitamin D supplementation using VDR genotyping in vitamin D insufficient KOA patients.

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TOWARDS A MOLECULAR DESCRIPTION OF DISEASE; A SYSTEMS BIOLOGY APPROACH IN OSTEOARTHRITIS

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Purpose: Osteoarthritis represents an increasing threat worldwide, affects people of all ages, inducing premature disability and economic impairment. Bioinformatics, computational and systems biology are used for defining molecular profile of diseases by integrating available information. This approach requires knowledge about details as well as overview about predictable interconnections. Bioinformatics is proposed as a tool in organizing existent knowledge aiming introduction of the discovery science principles in handling clinical and experimental data. Computational biology is used to detect possible correlations and links in molecular pathways and further pointed as one useful method to design focused systematic research.
Methods: Genes and proteins involved in the process specific to Homo sapiens have been extracted using data available in Gene Cards, Uniprot, pubmed cited literature and gene ontology (GO) databases. Interactions network analysis was build using Gene Regulatory Network (GRN) and Gene Set Enrichment Analysis (GSEA) considering the following factors; down and up regulation, co expression, predicted protein interaction, Transcription Factor (TF) regulation. ChIP-seq data sets were aligned usin to build version version hg19 of the human genome. Enhancers were defined as regions of ChIP-seq enrichment for H3K27ac in human cells.
Results: Several sets of genes previously associated with chondrocyte metabolism and inflamation were identified. Gene expression not previously associated with OA have been identified. OA-associated SNPs appear in super-enhancers of chondroprogenitor cells.
Conclusion: systems biology approach in disease characterization has the potential in defining the pathological mechanisms, in offering biomarker and therapeutical targets. Our approach is acontribution in this respect towards a definition of osteoarthritis.

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IMPROVED PREDICTION OF KNEE OSTEOARTHRITIS PROGRESSION BY GENETIC POLYMORPHISMS. ARTHROTEST STUDY RESULTS

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Purpose: Single Nucleotide Polymorphisms (SNPs) are inherited genetic variations that can predispose or protect individuals against clinical events. Osteoarthritis (OA) has a multifactorial etiology with a strong genetic component. Genetic factors influence not only knee OA onset, but also disease progression. The aim of this study was to develop a genetic prognostic tool to predict radiologic progression towards severe disease in primary knee OA (KOA) patients.
Methods: Cross-sectional, retrospective, multicentric, association study with Spanish KOA patients. 595 patients from 31 sites were selected. Inclusion criteria: Caucasian patients aged ≥40 years at the time of diagnosis of primary KOA, for whom two anteroposterior X-rays were available, one corresponding to the time of OA diagnosis with Kellgren–Lawrence grade 2 or 3 and the other to the end of the follow-up period. Patients who progressed to KL score 4 or were referred for total knee replacement in ≤ 8 years since the diagnosis were classified as progressors to severe disease. A unique expert viewer measured the radiologic progression from all X-rays. A candidate gene study analyzing 774 SNPs was conducted. SNP genotyping was performed with Illumina Golden gate technology or KASPar chemistry. Clinical variables of the initial stages of the disease (gender, BMI, age at diagnosis, OA in the contralateral knee and OA in other joints) were registered as potential predictors. Univariate analysis was done to identify associations between the baseline clinical variables or SNPs and KOA progression. SNPs and clinical variables with an association of $p < 0.05$ were included on the multivariate analysis using forward logistic regression.
Results: 282 patients fulfilled DNA and X-ray quality control criteria (220 in the exploratory cohort and 62 in the validation cohort). The univariate association analysis showed that one of the clinical variables and 23 SNPs were significantly associated to KOA severe progression in the exploratory cohort ($p < 0.05$). The predictive accuracy of the clinical variable was limited, as indicated by the area under the ROC curve (AUC = 0.66). When genetic variables were added to the clinical model (full model) the prediction of KOA progression was improved and the AUC increased to 0.82. Combining only genetic variables, a predictive model with a good accuracy (AUC = 0.78) was also obtained. The predictive ability for KOA progression of the full model was confirmed on the validation cohort (two-sample Z-test $p = 0.190$).
Conclusions: Genetic polymorphisms predict radiologic progression more accurately than clinical variables. An accurate prognostic tool to predict primary KOA progression has been developed, based on genetic and clinical information from OA patients. This model could help clinicians optimize patients' preventive and therapeutic care, according to their OA progression rate, and personalize disease management.

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FUNCTIONAL ANALYSIS OF THE OSTEOARTHRITIS SUSCEPTIBILITY LOCUS MARKED BY THE POLYMORPHISM rs10492367

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Purpose: The arcOGEN genome-wide association study, published in 2012, reported that the rs10492367 G to T single nucleotide polymorphism (SNP) marks a region on chromosome 12p that is associated with hip osteoarthritis (OA) in Europeans, with an odds ratio (OR) of 1.14 and a p-value of 1.48×10^{-8}. rs10492367 is an intergenic SNP located 59 kb downstream of kelch domain-containing protein 5 (KLHDC5) and 96 kb downstream of parathyroid hormone-like hormone (PTHLH). While there is sparse published data for KLHDC5, PTHLH has been widely studied, with its protein having a crucial role in endochondral ossification. As rs10492367 is not in high linkage disequilibrium (LD) with any common non-synonymous transcript polymorphisms, it is unlikely that the OA association is due to a change in the coding sequence of either of these two nearby genes. Instead, rs10492367 could function as a cis-acting polymorphism, influencing PTHLH or KLHDC5 gene transcription, resulting in allelic expression imbalance (AEI). Furthermore, as rs10492367 resides in a region predicted to have enhancer activity based on the ENCODE dataset, identification of functional variants within this region that affect such activity will prove vital in dissecting the OA signal. Thus, the aims of this study are to assess whether rs10492367 marks AEI in either PTHLH or KLHDC5, and to determine if any intergenic SNPs in high LD (≥0.8) with rs10492367 are functionally active in regulating enhancer activity of this OA associated region.
Methods: Cartilage was obtained from the hip or knee of OA patients who had undergone elective total joint replacement, and from non-OA patients who had undergone total joint replacement as a result of a neck-of-femur fracture (NOF). Using quantitative real-time polymerase chain reaction (qPCR), overall expression of PTHLH and KLHDC5 was measured in cDNA synthesised from cartilage RNA and stratified by rs10492367 genotype. Allelic expression imbalance analysis was performed using pyrosequencing. Since rs10492367 is intergenic, the transcript SNPs rs6253 in PTHLH and rs9029 in KLHDC5 were used as markers to measure allelic imbalance in 29 and 21 heterozygous OA patients, respectively. The data was stratified by rs10492367 genotype to evaluate whether any allelic differences corresponded to the presence of either of the association SNP alleles. Eight SNPs were in an LD of ≥0.8 with the association SNP; thus, 18 pGL3 promoter vector constructs were generated, each of which contained one of the alleles from

the nine SNPs. Luciferase reporter assays were used to assess if the presence of any of the alleles influenced the enhancer activity of the constructs in the U2OS osteosarcoma cell line, which expresses both genes.
Results: There was a significant increase in PTHLH expression in OA females relative to NOF females ($p = 0.02$), however there was no evidence for any significant correlation between PTHLH expression and rs10492367 genotype in OA cartilage. Similarly, there was no correlation between KLHDC5 expression and rs10492367 genotype. In addition, although allelic imbalance was observed, it did not correlate with the OA association SNP genotype. In the U2OS cell line luciferase assays, the OA associated alleles of the intergenic SNPs rs11049206 and rs58649696 resulted in significantly reduced enhancer activities relative to the non-OA alleles ($p = 0.0025$ and $p > 0.0001$, respectively).
Conclusions: In OA cartilage, our data does not support an association of rs10492367 with OA by mediating its effect on PTHLH or KLHDC5 expression. This may be due to the current analysis being limited to the aforementioned genes in end stage OA cartilage, as the OA associated region may be exerting its effects on other genes, in other tissue types or at different stages of development. However, luciferase reporter assays in an osteosarcoma cell line identified two intergenic SNPs in high LD with the associated SNP rs10492367 that independently resulted in reduced enhancer activity when the OA associated alleles were present. This current research therefore highlights the functional activity of the OA associated region marked by rs10492367.

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ADIPOSE TISSUE ASSOCIATED GENES IN HAND OSTEOARTHRITIS IN FINNISH WOMEN

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Purpose: Osteoarthritis (OA) is the most common joint disorder, the joints of the hand being the most frequent site affected. Available evidence suggests that genetic factors may play a major role in the etiology of OA. We chose to analyze in our hand OA material 21 single nucleotide polymorphisms (SNPs) from 10 adipose tissue associated genes (FTO, LEP, LEPR, ADIPOQ, RETN, NAMPT, SERPINA12, ITLN, RARRES2 and APLN) and their association with OA.
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: Association of studied SNPs to hand OA phenotypes were found from LEPR, RARRES2, RETN and APLN genes. FTO and INTL SNPs had borderline significant associations to hand OA phenotypes. Principle component analysis resulted in 11 principle components weighted by their genetic scores. Component 4 including four ADIPOQ SNPs, component 6 including RARRES2 SNP, and component 8 including APLN SNP had association to hand OA phenotypes.
Conclusions: Our results suggest that ADIPOQ, LEPR, RARRES2, RETN and APLN genes may play role in hand OA etiology in Finnish women.

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THE OSTEOARTHRITIS ASSOCIATION MARKED BY SNP rs6094710 MEDIATES ITS EFFECT BY REDUCING THE EXPRESSION OF NCOA3 IN JOINT TISSUES

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Purpose: Recently it has been reported that the rare single nucleotide polymorphism (SNP) rs6094710 is associated with hip osteoarthritis (OA) in European populations. The SNP is a G/A transition, with the A-allele having a frequency of 4%. The A-allele is more common in OA cases versus controls and this association reached genome-wide significance, with a p-value of 7.9×10^{-9} and odds ratio of 1.28. rs6094710 is intergenic, maps to chromosome 20q12 and is located upstream of the gene NCOA3, which codes for nuclear receptor co-activator 3. This protein interacts with nuclear hormone receptors and has histone acetyltransferase activity. Prior to the genetic study there were no reports of this protein having a role in OA. rs6094710 is in perfect linkage disequilibrium with rs6094752, a missense polymorphism leading to an amino acid change at position 218 of the NCOA3 protein. However, scrutiny of a variety of public databases reveals that this substitution is benign. This, combined with the absence of other protein-coding changes, make it probable that the 20q12 association mediates its affect by modulating gene expression. The aim of this study therefore was to investigate the effect of genotype at rs6094710 on the expression of NCOA3.
Methods: DNA and RNA were extracted from the cartilage of OA patients who had undergone elective joint replacement surgery. DNA was used to genotype patients at rs6094710. cDNA was synthesized from the RNA. Using real-time quantitative PCR (qPCR) we initially assessed whether genotype at rs6094710 correlated with overall expression of NCOA3. We next assessed allelic expression imbalance (AEI) of NCOA3, using the transcript SNP rs6094752 as a perfect proxy for rs6094710. AEI was measured in heterozygotes by pyrosequencing, with DNA providing the 1:1 ratio to which the cDNA ratios were compared. We have previously generated gene expression microarray data for hip cartilage from patients who had undergone joint replacement surgery due to either OA ($n = 11$) or a neck-of-femur (NOF) fracture ($n = 13$). This latter cartilage serves as a non-OA control. We used this data to compare the expression of NCOA3 between OA and non-OA hip cartilage.
Results: We performed qPCR analysis on the cartilage cDNA of 47 OA patients and observed that overall NCOA3 expression correlated with genotype at rs6094710 ($p = 0.021$; t-test). Reduced expression of NCOA3 was observed in individuals carrying a copy of the OA associated A-allele. AEI analysis confirmed the reduced expression from this allele, which produced an average of 37% less transcript than the G allele ($p < 0.001$; t-test). This AEI was also observed in fat pad (also an average 37% reduction) and synovial membrane (51% reduction) from OA patients, and in both males and females. Analysis of our microarray data revealed that NCOA3 is up-regulated 1.27 fold ($p = 0.004$; Mann–Whitney U test) in OA hip cartilage relative to control NOF cartilage.
Conclusions: Our results indicate that the OA association signal marked by rs6094710 is mediating its effect through a reduced expression of the gene NCOA3. Based on our microarray data we hypothesize that NCOA3 expression needs to increase to attenuate the OA disease process but that this increase is hampered in individuals carrying the A-allele of rs6094710, which therefore acts as an OA risk allele. Much more detailed functional studies of the gene and its encoded protein are now merited.

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KNEE OSTEOARTHRITIS GENETICS IN FINNISH HEALTH 2000 SURVEY

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Purpose: The aim of the study was to elucidate the genetic background of osteoarthritis (OA) and its correspondence to the genetics of overweight and metabolic syndrome (MetS) in Finnish population. OA and MetS share age and obesity as risk factors, and they both have been considered as an inflammatory disease in which different inflammatory mediators (for example cytokines, lipid derivatives, reactive oxygen species) are released by cartilage, bone and synovium. Obese patients with MetS have higher risk of developing OA than obese patients without MetS. Investigators are now assessing how MetS as a whole is linked to OA, and some have already nominated 'metabolic OA' as the fifth component of MetS. Genome-wide association studies (GWAS) have identified several single nucleotide polymorphisms (SNPs) associated with obesity and MetS. One of the most investigated genes has been FTO (fat mass- and obesity-associated) that regulates appetite. Genome-wide association studies have identified that polymorphisms in the FTO gene are associated also with risk of OA.
Methods: We chose to analyze 58 SNPs from 45 genes and their association to knee OA in Finnish Health 2000 Survey material. The Health 2000 Survey was conducted in Finland between fall 2000 and spring 2001. It is a representative sample of the Finnish population ($n = 8028$). The subjects were aged between 30 and 99. Knee osteoarthritis was diagnosed in 6.1 % of men and 8.3 % of women. For the genetic analyses