GENOME-WIDE ASSOCIATION SCAN OF OSTEOARTHRITIS OF THE KNEE

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Purpose: Osteoarthritis (OA) of the knee has an increasing public health impact in aging populations. Combinations of genes have shown potential to identify individuals at high risk of disease. However, to date the discovery of novel genetic risk factors for knee OA using large-scale genome-wide association methods has not been undertaken.

Methods: In this study the allele frequencies of 550,000 markers from the HumanHap550 Whole-Genome Genotyping from pooled genomic DNA from 713 female knee OA cases and 569 controls were calculated. Data for markers in common with the HumanHap300-Duo Genotyping BeadChip were combined with individual genotypes for 100 knee OA cases and 500 controls (all female).

Results: Preliminary analyses indicated that genes part of pro-apoptotic signaling, in the Wnt signaling and Smad signaling pathways that had not previously shown to be involved in OA were associated with disease risk. For the ten markers with the smallest \(p\)-values, all showing genome-wide significance, two or more SNPs in the same gene region were selected and genotyped individually for the original 813 cases plus additional 540 cases and for the original 1069 controls plus additional 1040 controls.

Conclusions: We present the combined results of the discovery and replication samples in the largest genetic association study of knee OA in women to date.

ASSOCIATION OF GDF5 WITH OSTEOARTHRITIS AND ITS MOLECULAR PATHOGENESIS

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Purpose: Osteoarthritis (OA) is the most common bone and joint disease. Genetic factors have been implicated in its etiology and pathogenesis. Growth and differentiation factor 5 (GDF5) is a growth factor that belongs to a subgroup of the TGF-\(\beta\)/BMP family. GDF5 has been implicated in chondrogenesis, joint formation and OA. The aim of the study is to clarify the role of GDF5 in the etiology and pathogenesis of OA using combined approach of human and mouse genetics.

Methods: Human study. A case-control association study on GDF5 was performed for OA of the knee and hip joints in Japanese using several independent cohorts including a total of more than 3,500 subjects (1,000 hip OA, 718 knee OA and 1,845 control). A susceptibility gene was located by a linkage-disequilibrium mapping. The replication of the association was examined in a Han Chinese knee OA population. The allelic difference of the associated sequence variation was evaluated by \textit{in vitro} functional studies.

Mouse study. Through a large-scale ENU mutagenesis screen, a mouse mutant with Gdf5 mutation was identified. This mouse carries an amino acid substitution (W408R) in a highly conserved...
region of the signaling domain of Gdf5 protein. The phenotype of the mouse was characterized by radiographic and histological examination. The mechanism of the mutation was examined in vitro using recombinant Gdf5s.

**Results:** Human study. GDF5 is associated with OA. A single nucleotide polymorphism (SNP) in its 5’-UTR (+104T/C) showed a significant association (P=1.8 x 10^{-13}; odds ratio= 1.8) in two independent hip OA populations. This association was replicated for knee OA in Japanese and Chinese. Located in the GDF5 core promoter, this SNP exerted allelic differences on transcriptional activity in chondrogenic cells, with the susceptibility allele showing reduced transcriptional activity.

**Mouse study.** The mutation is semi-dominant, showing brachypodism and digit ankylosis in heterozygotes, and much more severe brachypodism, ankylosis of the knee, and early-onset OA of the elbow in homozygotes. The mutant GDF5 protein was secreted and dimerizes normally, but did not induce BMP signal nor cartilage matrix gene expression. It inhibited the function of the wild-type Gdf5 protein in a dominant-negative fashion.

**Conclusions:** GDF5 is a susceptibility gene for OA. Decrease of GDF5 is involved in OA pathogenesis and impairs joint maintenance. The Gdf5 mutant mouse would serve as a good model for OA.

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**A SNP IN THE 5' UTR OF GDF5 IS ASSOCIATED WITH OSTEOARTHRITIS SUSCEPTIBILITY IN EUROPEANS AND WITH IN VIVO DIFFERENCES IN ALLELIC EXPRESSION IN ARTICULAR CARTILAGE**

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**Purpose:** A compelling genetic association with OA of a functional SNP (rs143383, T/C) in the 5’ UTR of the GDF5 gene was recently reported in case-control cohorts from Japan and China. GDF5 is a pro-chondrogenic growth factor. The T-allele frequency of the gene was elevated in cases, with an odds ratio (OR) of 1.79, and in vitro functional studies demonstrated that this allele mediated a moderate but significant reduction in the activity of the GDF5 promoter in several cell lines. Our objectives were to assess whether the SNP was also associated with OA in a broad European population and to assess the functional effect of the SNP on GDF5 allelic expression using RNA extracted from the cartilage of OA patients who had undergone joint-replacement surgery.

**Methods:** The SNP was genotyped in 2487 OA patients (cases) ascertained by the need for joint replacement surgery of the hip or knee and in 2018 age-matched controls. RNA was extracted from the articular cartilage of 9 OA patients who were heterozygous at the SNP and was used to measure allelic expression by a single base-pair extension assay that can discriminate and quantify the mRNA synthesised from each allele.

**Results:** The T-allele was associated with OA (P = 0.03, OR = 1.10) as was carrier status for this allele (P = 0.004, OR = 1.28). The associated T-allele showed up to a 27% reduction in expression relative to the C-allele in OA cartilage (P = 0.00007).

**Conclusions:** Our study supports the Asian report and highlights GDF5 as a globally relevant OA susceptibility locus. The functional difference mediated by SNP rs143383 on GDF5 expression is active in patients who have severe end stage disease up to the point at which they require surgery, implying that a small but persistent imbalance of GDF5 expression throughout life renders an individual more susceptible to OA.

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COMMON GENETIC VARIATION IN THE ESTROGEN RECEPTOR BETA GENE IS ASSOCIATED WITH INCREASED RISK OF OSTEOARTHRITIS

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**Purpose:** To examine the relationship between common genetic variation of the ESR2 gene and radiographic hand-, knee-, hip- and generalized osteoarthritis (ROA) in two large population-based cohorts: the Rotterdam Study and the Chingford Study.

**Methods:** In total, 5222 subjects had data available on genotype and ROA outcomes. ROA was defined as a Kellgren/Lawrence score (K/L score) ≥2. Seven tagging SNPs were genotyped in the Rotterdam Study and one of these SNPs, rs1256031, was genotyped in the Chingford Study.

**Results:** In the Rotterdam Study, female homozygote carriers of the C allele of the rs1256031 SNP (htSNP2) had an 84% increased risk for hip OA (95% CI 1.17-2.87, p=0.008) and an 80% increased risk for generalized OA (95% CI 1.15-2.82, p=0.01). A 100% increased risk was observed for progression of hip OA (95% CI 1.24-3.23, p=0.005) and a non-significant trend towards higher levels of CTX-II was observed. Similar trends were seen in the Chingford Study, where an increased risk for hip-, knee- and progression of hip OA and higher CTX-II levels were observed, although not significant. Four common haplotypes were inferred from 7 tagging SNPs. Haplotype analysis confirmed the associations observed with single SNP analysis.

**Conclusions:** Female carriers of the C allele of the rs1256031 SNP of the ESR2 gene have an increased risk of OA. This SNP is in high linkage disequilibrium with 21 other SNPs, including 18 potential functional variants that could explain the increased risk for OA. This study showed that common genetic variation in the ESR2 gene influences the risk of osteoarthritis.