root for >3 months. We extracted genomic DNA from peripheral blood leukocytes of affected individuals and controls using standard protocols. Written informed consent was obtained from each subject.

Genotyping and Statistical Analysis.

We genotyped SNPs using the multiplex PCR-based Invader assay, TaqMan SNP genotyping assays, or by direct sequencing of PCR products. We assessed association and Hardy-Weinberg equilibrium using the χ2 test. We estimated haplotype frequencies using the expectation-maximization algorithm.

Functional Studies.

Allelic difference in the function of the associated SNPs was examined in vitro and in vivo according to their possible roles in pathogenesis of LDH and available experimental resources.

Results: We selected candidate genes based on our prior knowledge on their function, expression, animal models, and human monogenic diseases, etc. CILP (cartilage intermediate layer protein), ASPN (asporin), COL11A1, THBS2 (thrombospondine 2), MMP9 (matrix metaloprotease 9) and SKT (sickle tail) were found to be associated with LDH. CILP-susceptibility allele was a missense SNP whose predicted protein product had a stronger inhibitory effect on TGF-β function for cartilage differentiation. COL11A1-susceptibility allele showed decreased COL11A1 mRNA expression in vitro and in vivo. ASPN and SKT associations were replicated in different ethnic groups. THBS2-susceptibility allele was located in a polymyridine tract upstream of the 3’-splice site an intron 10 and exerted allelic differences on exon skipping rates in vivo, with the susceptibility allele showing increased skipping. The exon skipping resulted in decreased THBS2 interaction with MMP2 and MMP9.

Conclusions: Through a large-scale candidate gene approach, we have found several LDH-susceptibility genes, which would help in clarifying the pathogenesis of LDH and in developing innovative treatment for LDH.

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ASSOCIATION BETWEEN IL-4 RECEPTOR α AND TGF-β1 POLYMORPHISMS AND HAND OA

S. Hämäläinen, S. Solovieva, T. Vehmas, P. Leino-Arjas, A. Hirvonen

Finnish Inst. of Occupational Hlth., Helsinki, Finland

Purpose: Osteoarthritis (OA) is a common disease characterised by the degeneration of the cartilage of synovial joints such as the hip and knee. Available evidence suggests that genetic factors play a major role in etiology of OA. The gene product of IL-4 receptor (IL-4R) regulates cartilage chondrocyte differentiation and survival. Transforming growth factor (TGF)-beta, on the other hand, regulates the function of fibroblasts, and has been shown to have a role in the pathogenesis of rheumatoid arthritis. These enzymes may therefore play an important role in development of OA. Both IL-4R and TGF genes have been shown to exhibit genetic polymorphisms with functional consequences. We examined whether these gene polymorphisms modified individual susceptibility to hand OA in Finnish women.

Methods: Radiographs of both hands of 543 Finnish women aged 45-63 years were examined and classified for the presence of OA using reference images. Hand OA was defined by the presence of radiographic findings of grade 2 or more in at least two joint pairs (symmetrical OA) or in at least two DIP joint pairs (symmetrical DIP OA). The IL-4R Ser503Pro (rs1805015) and TGFβ1 Leu10Pro (rs1982073) genotypes were determined using TaqMan-based methods. Data regarding anthropometric measures and other risk factors were collected by questionnaire.

Results: No significant association was found between the IL-4R Ser503Pro polymorphism and hand OA. However, the TGF-β1 10Pro allele posed a 1.6-fold (95% CI 1.0-2.5) and a 1.8-fold (95% CI 1.0-2.6) risk of symmetrical OA and symmetrical DIP OA, respectively. Moreover, the risk of symmetrical OA was almost 6-fold (OR 5.6, 95% CI 1.3-24.7) among carriers of the combination of 4L-4R 503Pro and TGFβ1 10Leu alleles.

Conclusions: Our results suggest that the studied IL-4R- and TGFβ1-gene polymorphisms may play a role in the etiology of polyarticular hand OA.

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FUNCTIONAL ANALYSIS OF THE GROWTH AND DIFFERENTIATION FACTOR 5 REGULATORY POLYMORPHISM THAT IS ASSOCIATED WITH OSTEOARTHRITIS SUSCEPTIBILITY

R.J. Egli1,2, L. Southam1, J.M. Wilkins1, I. Lorenzen1, M. Pombo-Suarez3, A. Gonzalez3, A. Carr1, K. Chapman1, J. Loughlin4


Purpose: Over the past decade, a genetic component for the multifactorial disease osteoarthritis (OA) has been established. Among others, a consistent and reproducible association with OA was found for the single nucleotide polymorphism (SNP) rs143383 (T/C) in the 5’UTR of the growth and differentiation factor 5 (GDF5) gene with a lower expression of the risk-associated T-allele in vitro and in vivo, the latter in cartilage tissue from OA patients. To further characterize its role in OA susceptibility, we have expanded the analyses of the effect of this SNP on GDF5 allelic expression to more joint tissues, and searched for cis and trans factors that interact with the SNP.

Methods: Tissues (cartilage, fat pad, meniscus, synovium, ligaments) were collected from OA patients undergoing joint replacement of the hip or knee. Nucleic acid was extracted and, using rs143383 and an assay that discriminates and quantifies allelic expression, the relative amount of GDF5 expression from the T and C alleles was measured. Electrophoretic mobility shift (EMSA) and luciferase assays were used to search for trans factors regulating transcriptional expression of GDF5 at rs143383.

Results: We observed a consistent allelic expression imbalance of GDF5 with a reduced expression of the risk-associated T-allele of approximately 20% in all tissues tested, implying that the functional effect rs143383 mediates on GDF5 expression is joint-wide. A differential binding of transcription factor(s) to rs143383 was revealed by EMSA. Among putative candidates tested, DEAF1 showed preferential binding to the T-allele of rs143383. Testing GDF5 promoter constructs in a luciferase assay confirmed DEAF1 as being involved in the transcriptional regulation of GDF5 with a stronger repression of the T-allele at rs143383. However, we identified a further polymorphism in the 5’UTR of GDF5 (rs143384), which strongly influenced transcriptional regulation and allelic expression at rs143383.

Conclusions: The OA susceptibility being mediated by polymorphisms in GDF5 is not restricted to cartilage, emphasising the need to consider the disease as involving the whole joint. DEAF1 is a trans-acting factor that merits further investigation as a tool for potentially modulating GDF5 expression, and the existence of an additional cis-acting regulatory polymorphism highlights the complexity involved in regulating the expression of this important OA susceptibility locus.