Role of HOX Family Transcription Factors in Regulating Hand Osteoarthritis Genetic Risk

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INTRODUCTION: Osteoarthritis (OA) is a heterogeneous disease that affects not only the weight-bearing joints, such as the hip and knee, but also the non-weight-bearing smaller joints in the hand at a high prevalence. The lifetime risk of developing symptomatic hand OA (hOA) in at least one hand by the age of 75 years is 40%, with women exhibiting twice the risk as men. Moreover, there is substantial evidence from the Million Veteran Program data that military service increases the incidence of hand/finger/thumb OA by 30-50%, implying that overuse and injuries (micro and macro trauma) of hand joints are a leading cause for hOA. Despite significantly increasing pain, disability, health costs, and caregiver dependence, hOA treatments are limited to generalized pain relief, mainly due to a limited knowledge of the underlying molecular mechanisms specific to hOA pathogenesis. In this study, we utilized data from multiple population-based genome-wide association studies (GWAS) to examine single-nucleotide variants (SNVs) that statistically associate with hOA. We uncovered that the HOX family transcription factors may play a crucial role in determining the genetic risk for hOA pathogenesis.

METHODS: We leveraged a recent GWAS study consisting of individuals from the Million Veteran Program and the UK Biobank, along with small single population-based GWAS risk assessment studies for hOA to compile a comprehensive list of the SNVs that specifically associated with a high risk for hand OA development. We then utilize genomic data analysis tools available on ENCODE to predict the potential transcription factor binding sites in proximity to the hOA SNVs. We further explored publicly available ChIP-seq data for transcription factors that are associated with an increased hand OA risk. Based on these bioinformatic analyses, we predicted the transcriptional regulatory networks that specifically govern the inflammatory and degenerative pathology of hand OA in humans. We validated the hand OA-specific association of the predicted transcription factors via gene expression knockdown and ChIP experiments from synovial fibroblasts derived from the finger joints of hOA patients and arthritic mouse paw joints.

RESULTS: Our Genome-wide association studies (GWAS) evaluation predicted up to 22 single-nucleotide variants (SNVs) that increase the risk of developing hOA. These SNVs were located in non-coding regions in proximity to 14 potential hOA risk candidate genes. These candidate hOA risk genes included the previously established hOA risk SNVs located at the *ALDH1A2* gene locus as well as multiple other genes, including *CYP26B1*, *RBFOX1*, *IRF2BP1*, *TEAD1,* and *ASTN2,* whose role in hOA pathogenesis remains to be determined. Interestingly, our transcription factor DNA binding motif predictions revealed the occurrence of HOXA13 and HOXD13 binding sites close to the SNVs, suggesting a potential role for the HOX patterning factors in determining hOA risk. Furthermore, supporting this finding, our analysis of published HOXA/D13 ChIP-seq and RNA-seq from mouse E11.5 limb buds revealed that these transcription factors regulate the expression of multiple hand OA risk candidate genes. Finally, our *Hoxa/d13* shRNA knockdown experiments in mouse paw vs hip synovial fibroblasts indeed confirmed that the hOA risk target genes are regulated by HOXA13 and D13 transcription factors.

DISCUSSION: HOXA and D13 are paralogous genes arising from gene duplication during evolution. They encode transcription factors that bind the same DNA motif. These genes are critical components of the skeletal patterning genes and are indispensable for the distal limb skeletal domain development, which includes the hands/paws in higher vertebrates. Our studies suggest that the anatomical location undoubtedly matters in defining transcriptomic features of each joint and that the unique location-specific features can have a major influence on the diagnosis, prognosis, and treatment of OA. Importantly, our studies show that the HOX code, which establishes the limb skeleton pattern during embryonic development, persists in the adult joints, and that the HOX code likely governs the differential physiological and pathological properties of each joint. Therefore, understanding the role of HOX code in OA FLS will drive the development of anatomical site-focused OA therapeutic solutions.

SIGNIFICANCE: It is well established that the etiology of hOA is distinct from load-bearing hip and knee OA. Therefore, the repair/resurfacing approaches being actively pursued for fixing cartilage defects in hip or knee OA are unlikely to be applicable for hOA. Our limited understanding of the molecular mechanisms that distinguish joint-specific disease features is also contributing to the deficits encountered in current treatment approaches for OA. In conclusion, hOA largely remains a neglected aspect of chronic joint pain, and progressive loss of function requires urgent attention and our approach to leverage the HOX code memory to identify new targets for hOA-focused therapeutic approaches is conceptually innovative.