Identifying Genes Associated with Degenerative Disc Disease Using an Inbred Mouse Aging Panel

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INTRODUCTION: Causing up to 5% of all lower back pain (LBP) cases, Degenerative Disc Disease (DDD) presents significant burden to an estimated 100 million patients worldwide. Up to 30% of DDD patients cannot be managed by pain medication, necessitating surgical interventions like microdisectomy, or spine fusion. Both procedures have high relapses and/or revision rates. While DDD is part of the natural aging process, early or rapid intervertebral disc degeneration is highly heritable, yet a clearly delineated genetic basis remains poorly understood. As diagnosis of DDD requires MRI evidence of disc degeneration, the cost involved in data collection for large human genetic studies can be prohibitive. An inbred mouse aging panel offers distinct advantages including cost effectiveness and genetic homogeneity within strains allowing for repeated measures of phenotype. In addition, the strains used have all been sequenced and annotated. Together these factors provide a more tractable and accurate system for identifying candidate genes associated with DDD. Genome Wide Association Studies (GWAS) identify statistical associations between naturally occurring genetic variation and phenotypic traits. When applied to the inbred mouse aging panel, GWAS leverages controlled breeding and uniform environments to increase statistical power and reduce confounding diseases found in humans. This study combined GWAS methodology and inbred mouse aging panel in order to uncover the genetic profile of DDD in humans.

METHODS: **[Inbred Mouse Aging Panel Phenotyping]** Male and female mice of 25 inbred mouse strains from groups at 12-month, 20-month, and >20-month of age (sampled at death) were used. These ages of sampling are approximate to human ages of 40-years-old, 70-year-old and 70-year-old and beyond respectively. In total, 1,259 mice were included in this study. The entire spine was decalcified, paraffin embedded, sectioned, and stained with H&E. Sections containing discs were graded by two board-certified veterinary pathologists (JPS, JMW) and scored for rupture using a semiquantitative system: 1s for the presence of disc degeneration and 0s for absence of disc degeneration. **[GWAS]** GWAS was performed using the Efficient Mixed-Model Association (EMMA) algorithm, which accounts for population structure by incorporating kinship matrix into a linear mixed model. Association between genetic polymorphisms and disc degeneration was computed, with statistical significance assessed using *p*-values. Loci were identified as having a *p*-value that exceeded a Bonferroni-corrected threshold. Confidence intervals were defined using a 2 logarithm of the odds (LOD) score drop and expansion by 1 million base pairs up and downstream of the peak. Genes within these loci were tested to determine if allelic differences within the gene met the allele effects of the association. **[Immunofluorescence, IFC]** Immunofluorescence (IFC) on sagittal sections of intervertebral disc tissue from healthy and DDD-affected mice was used to assess ING3 expression distribution, the protein encoded by the top GWAS-identified gene. A primary antibody against ING3 and a fluorescently labeled secondary antibody enabled visualization of its localization within the disc.

RESULTS SECTION: **[Inbred Mouse Aging Panel Phenotyping]** Phenotyping analysis of the inbred mouse panel showed various degrees of naturally occurring disc degeneration frequency (incidence is number of new cases per 100,000 population, which was not done here) across 3 age groups and between male and female groups: 13% in 12-month female, 19% in 12-month male, 38% in 20-month female, 44% in 20-month male, 39% in >20-month female, 30% in >20-month male. **[GWAS]** The GWAS revealed both sex- and age-dependent differences in the association between genetic polymorphisms and disc degeneration. Notably, genetic variants exhibited stronger associations in male groups compared with female groups. Using the Bonferroni corrected threshold, 24 loci were identified in the 12-month male group, 4 in the 20-month male group and 1 in the >20-month male group. No loci were identified in females suggesting a strong gene*sex interaction. No overlap of loci was found across the 3 male age groups, highlighting heterogeneous genetic architecture of DDD at different ages. *Ing3* was identified as a candidate gene in 12-month-old male mouse for a locus at 6qA3.1, corresponding to 7q31.31 in human; the most significant locus identified in this study (P< 2.07E-19). **[IFC]** IFC for *Ing3* showed diminished or absent expression in the annulus fibrosis and endplate cells adjacent to ruptured discs, whereas healthy controls displayed strong expression in these regions. **[Functional analysis]** Besides *Ing3*, 66 genes additional putative candidate genes in the 12-month male groups, 10 in the 20-month male group and 4 in the >20-month male group were identified. Many of these genes had been previously reported using GWAS for DDD. Gene set enrichment analysis of candidate genes showed over representation of genes associated with cartilage development, positive regulation of stem cell differentiation, regulation of chondrocyte differentiation, osteoblast differentiation, and positive regulation of ossification.

DISCUSSION: Previous studies of mice have suggested that DDD was a rare occurrence in this species, but these studies were restricted to only a few strains and usually were conducted in very young mice. This study demonstrates that mice do develop spontaneous disc degeneration, with frequencies comparable to those observed in humans. Although results of this study suggest stronger genetic predisposition of DDD in males and biobank studies showed higher percentage of DDD incidence in men, clinical data shows that more women received treatment for DDD. Our work provides a new avenue to examine gene*sex interactions underlying DDD. Since this study used the most stringent correction of significant threshold, no loci were identified in female groups. With a more lenient threshold, we did identify loci in female, but these loci were suggestive only. Previous studies of *Ing3* showed that it is involved in pathways that were associated with DDD, suggesting potential mechanistic relevance deserving further inquiry in cell line or human cohorts.

SIGNIFICANCE/CLINICAL RELEVANCE: This study offers insight into the gene etiology of DDD. Moreover, this study proposes strong candidate genetic markers for hereditary DDD, which may lead to future therapeutics or the ability to test for risk. The latter could allow for the implementation of early (pre-disease) interventions.

IMAGES AND TABLES:

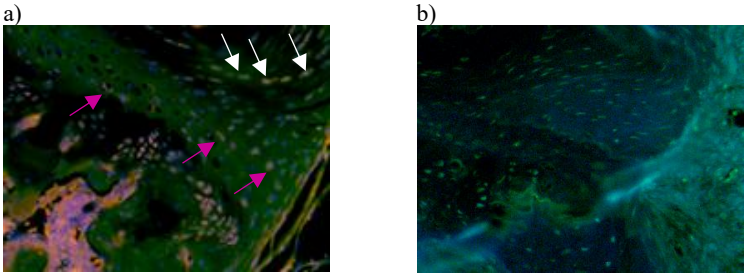

Figure 1. IFC of healthy and DDD discs. Bright yellow signal indicate expression of ING3.

a) IFC of sagital spine section of healthy mouse. White arrows indicate ING3 expression in the annulus fibrosis and purple arrows indicate ING3 expression in the endplate.

b) IFC of sagital spine section of DDD mouse with no expression of ING3.