Genome wide association study identifies potential genetic polymorphisms underlying the arthrofibrosis phenotype and suggests previously unexplored biological mechanisms

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INTRODUCTION:

Arthrofibrosis (AF), characterized by excessive periarticular scar tissue formation resulting in joint stiffness and soft tissue contracture, is one of the most complex complications after primary total knee arthroplasty (TKA) and total hip arthroplasty (THA)[1,2]. Despite AF being a well-documented complication of TKA, the pathogenesis of arthrofibrosis is incompletely understood, limiting the success of treatment methods[1]. Ultimately, patients who fail after AF treatments may need to undergo revision surgery, which has limited effectiveness in improving function and pain[6,8]. Genetic studies on keloids have identified differential expression of genes, such as secretory leukocyte protease inhibitor (SLPI), in affected cohorts, suggesting that the etiology of AF could lie in fibroblast-mediated healing. Enrichment analysis in genes from a small sample of patients shows promise in elucidating the molecular pathology of AF, however no studies have attempted to blindly identify genetic variants responsible for the AF phenotype, which could elucidate its plausible association with healing disorders and identify pathways previously unexplored. In this study, we performed a logistic genome-wide association study to identify genetic variants and relevant biologic pathways associated with the arthrofibrosis phenotype after total joint replacement.

METHODS:

A cohort of 1,609 adults who had undergone hip or knee arthroplasty and experienced stiffness complications were identified from a large genetic biobank. These cases were matched by demographics, comorbidities, and characteristics to a 2:1 control group of 3,218 patients who underwent arthroplasty without arthrofibrosis. A logistic genome wide association study (GWAS) was performed using plink2.0. The model quantified low-frequency variants with significant log-odds effect on the affected (case) phenotype and unaffected (control) phenotype. Significant SNPs were reported after applying a minor allele frequency threshold of 0.01. Population stratification among the genomes, due to differences in ancestry, was controlled via the inclusion of 10 principal components as covariates. Sex, age, body mass index (BMI), and affected joint for each individual were also accounted for as covariates. Bonferroni correction and Benjamin-Hochlberg false discovery rate (FDR) thresholds were used to correct p-values for multiple comparisons.

RESULTS:

Our results indicate that there are increased odds of expressing the arthrofibrosis phenotype after total joint replacement when there are mutations in or near the following six loci: ICA1 (OR[95%CI]: 2.04 [1.81-2.28]), AVEN (OR[95%CI]: 1.74 [1.55-1.92]), SYNC (OR[95%CI]: 1.65[1.48-1.83]),CAMK2D (OR[95%CI]: 1.51 [1.37-1.65]), GRM7 (OR[95%CI]: 1.52 [1.38-1.67]) and GRIK4 (OR[95%CI]: 1.44[1.33-1.55]). Investigation into the accompanying biological pathways supports an over-proliferation etiology in patients experiencing AF. Islet Cell Autoantigen 1 (ICA1) is a transport gene identified by gene set enrichment analysis studies to be involved in transport and neurotransmitter secretion. The Apoptosis and Caspase Activation Inhibitor gene (AVEN) has been found to be up-regulated at the active margin of keloids. Mutations in Syncoilin, Intermediate Filament Protein (SYNC) has been associated with myofibrillar myopathy type 1 and cytoskeleton deformities. Calcium/Calmodulin Dependent Protein Kinase II Delta (CAMK2D) has been shown to have a role in hypertrophic scar formation and to be upregulated with mechanical compression. Pathway analysis studies have shown Glutamate Metabotropic Receptor 7 (GRM7) to be vital for neurodevelopment and to be upregulated in high density fibroblasts in dense breast cancer stroma.

DISCUSSION AND CONCLUSION: This study entailed a step towards the creation of a *de-novo* gene signature for arthrofibrosis and the elucidation of its biological mechanism. Defining gene mutations associated with increased odds of arthrofibrosis post-arthroplasty allows to create a framework for additional pathway and enrichment analysis to identify previously unexplored biochemical mediators of AF and potential therapeutic targets. In recent years, pharmacologic treatments targeting the fibrotic cascade at the molecular level have become more common. However, long term follow-up studies for conditions related to connective tissue over-proliferation such as Dupuytren's contractures have reported that there is a high long-term recurrence after treatment. Our study indicates that there are potential alternative target pathways to that of collagen deposition. Moreover, CAMK2D presents a potential paradigm shift in treatment, as recent studies have shown it is responsive to mechanical stimuli.