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Evaluating whole-genome expression differences in idiopathic and diabetic adhesive capsulitis

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Abstract

Background: Diabetic patients have a greater incidence of adhesive capsulitis (AC) and a more protracted disease course than patients with idiopathic AC. The purpose of this study was to compare gene expression differences between AC with diabetes mellitus and AC without diabetes mellitus.

Methods: Shoulder capsule samples were prospectively obtained from diabetic or nondiabetic patients who presented with shoulder dysfunction and underwent arthroscopy (N = 16). Shoulder samples of AC with and without diabetes (n = 8) were compared with normal shoulder samples with and without diabetes as the control group (n = 8). Shoulder capsule samples were subjected to whole-transcriptome RNA sequencing, and differential expression was analyzed with EdgeR. Only genes with a false discovery rate < 5% were included for further functional enrichment analysis.

Results: The sample population had a mean age of 47 years (range, 24–62 years), and the mean hemoglobin A_{1c} level for nondiabetic and diabetic patients was 5.18% and 8.71%, respectively. RNA-sequencing analysis revealed that 66 genes were differentially expressed between diabetic patients and nondiabetic patients with AC whereas only 3 genes were differentially expressed when control patients with and without diabetes were compared. Furthermore, 286 genes were differentially expressed in idiopathic AC patients, and 61 genes were differentially expressed in diabetic AC patients. On gene clustering analysis, idiopathic AC was enriched with multiple structural and muscle-related pathways, such as muscle filament sliding, whereas diabetic AC included a greater number of hormonal and inflammatory signaling pathways, such as cellular response to corticotropin-releasing factor.

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Supplementary Data

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Conclusions: Whole-transcriptome expression profiles demonstrate a fundamentally different underlying pathophysiology when comparing diabetic AC with idiopathic AC, suggesting that these conditions are distinct clinical entities. The new genes expressed explain the differences in the disease course and suggest new therapeutic targets that may lead to different treatment paradigms in these 2 subsets.

Level of evidence: Basic Science Study; Molecular Biology

Keywords

Frozen shoulder; diabetes; RNA sequencing; adhesive capsulitis; whole genome sequencing; inflammation; corticosteriods

Adhesive capsulitis (AC), otherwise known as “frozen shoulder,” is a common affliction, affecting 2%−5% of the general population.[21] The disease process of AC is known to include fibroproliferative changes in the shoulder associated with capsular inflammation causing pain and limitation in motion, particularly external rotation. Magnetic resonance imaging and arthroscopic visualization have consistently characterized a thickening and irritation of the rotator cuff interval among patients with this disorder.[47] Some studies have also suggested the possibility of a chondrogenic or neuro-dystrophic etiology for AC.[15]

The underlying pathophysiology of AC is not yet completely understood, and treatments for the condition have remained relatively unchanged and continue to be chiefly marginally effective. Patients generally continue to be treated with physical therapy and often endure years of limited motion and pain.[31] Frequently, this conservative approach is accompanied by permanently reduced range of motion. Previous studies of human capsular samples of AC have led to inconsistent histologic results,[9–11,19,27,29,35,50,52] making consensus characterizing the disease difficult to achieve.[3,12,33] Cell types, such as the myofibroblast, and specific signaling pathways have been identified in numerous studies, with heterogeneous findings[1,9,13,21,29,30,32,37,40,47,52]; notably, the inciting events of the underlying condition remain incompletely understood.[3,12,33] Furthermore, previous studies have not pointed to a unified homogeneous disease process as results have varied with treatment. It is clear that thickening and irritation of the joint capsule occur and become typically unpredictably responsive to anti-inflammatory treatments such as nonsteroidal anti-inflammatory drugs or corticosteroid injections.[10,41] In the most protracted cases, symptoms continue for months or years and may result in permanent disability.[49]

Although AC most commonly presents with no precisely attributable etiology (idiopathic AC), several risk factors have been described in the literature. AC more commonly occurs in patients with thyroid disorders,[14] autoimmune dysfunction,[8] serum lipid alterations,[43] and Parkinson disease,[45] as well as other fibroproliferative diseases such as Dupuytren contracture or Lederhosen disease.[33,45,51] Notably, patients with diabetes and associated conditions such as hyperglycemia and metabolic syndrome have a much higher prevalence of AC compared with the general population.[5,36] The degree of hyperglycemia correlates with both the prevalence and the prognosis of AC, with patients with insulin-dependent diabetes presenting with AC at appreciably greater rates than patients receiving oral hypoglycemic agents.[53] Similarly, diabetic patients, especially those with poorly controlled

diabetes, fare much less well with nonoperative management than idiopathic AC patients.[46] Diabetic patients in whom AC develops also typically experience a more protracted disease course, often with failure of conservative management after months or years of physical therapy and the eventual need for arthroscopic capsular release.[31]

The purpose of this study was to compare genome-wide expression differences between AC with diabetes mellitus and AC without diabetes mellitus. Given that diabetes-associated AC generally presents with a more severe degree of stiffness and is less responsiveness to conservative treatment, we hypothesized that idiopathic AC and diabetic AC are distinct clinical entities with unique underlying pathophysiology, even though the histologic and gross appearance of tissue may be similar for both types of AC.[2] Understanding the pathophysiology of AC is essential to direct more targeted treatments of this widespread condition, which affects many patients. Our hypothesis is supported by previous clinical findings that have shown differential outcomes of AC based on patient risk factors. We used RNA sequencing to compare genome-wide expression profiles for patient samples taken from AC patients and control patients both with and without carrying a confirmed diagnosis of diabetes mellitus. Our study focuses on a profoundly important clinical problem for many patients and reveals new transcriptional differences in these disorders that notably could translate readily into clinical practice paradigms.

Materials and methods

Study design

Thirty-five patients presenting with shoulder pain or dysfunction to a large academic medical center and undergoing subsequent arthroscopy were prospectively enrolled in this study from 2015 to 2018. The inclusion criteria consisted of patients with AC undergoing arthroscopy or patients without AC undergoing arthroscopy owing to shoulder dysfunction attributable to other glenohumeral pathology, such as a rotator cuff tear. AC was defined as dysfunctional shoulder pain with restricted active and passive range of motion without other contributing glenohumeral pathology seen during preoperative imaging or arthroscopy. The exclusion criteria were patients with a history of thyroid disease, those with a history of surgery for AC, and those with other pathology affecting the shoulder capsule.

In total, 16 patients were included for analysis in this study and were stratified by the presence or absence of diabetes, determined through review of the electronic medical record or preoperative A_{1c} screening if patients had relevant risk factors, such as a family history or obesity. All diabetic patients in this study had type 2 diabetes, and no patients had a history of type 1 (insulin-dependent) diabetes. Diabetic patients with AC (diabetic AC, n = 4) were matched with control diabetic patients without AC (n = 4) for analysis. Similarly, nondiabetic patients with AC (idiopathic AC, n = 4) were matched with nondiabetic control patients without AC (n = 4) for analysis.

Patient demographic characteristics

The patients had a mean age of 47 years (range, 24–62 years). The mean duration of AC for diabetic and nondiabetic patients was 9.5 months and 9.7 months, respectively. The

right shoulder was involved in 50% of diabetic AC patients and 75% of idiopathic AC patients. Diagnoses in control patients without AC included rotator cuff tear or sprain (n = 5), shoulder instability (n = 2), and arthritis (n = 1). When patients with AC were compared with control patients without AC, 7 of 8 patients with AC had received at least a single intra-articular steroid injection vs. 2 of 8 patients without AC (Supplementary Table SI).

The mean hemoglobin A_{1c} level for nondiabetic and diabetic patients was 5.18% and 8.71%, respectively. Diabetic patients with and without AC had a mean duration of diabetes of 9.1 years and 11.1 years, respectively. The mean A_{1c} level of diabetic patients with and without AC was 7.8% and 9.6%, respectively.

Capsular tissue procurement

Shoulder capsular tissue was obtained from the rotator interval intraoperatively, with some portions of capsular tissue placed in ribonuclease-free microcentrifuge tubes, snap frozen in liquid nitrogen, and stored at −80°C. Remaining portions of capsular tissue were rinsed in sterile phosphate-buffered saline solution and fixed in 4% paraformaldehyde at 4°C for 4–5 days, followed by automatic tissue processing. Seven 1-μm-thick sections were stained with hematoxylin-eosin staining as well as safranin O with fast green counterstaining using routine procedures.

Tissue dissociation and RNA isolation and sequencing

Dissociation of capsular tissue for RNA extraction was performed using a customized protocol and the Miltenyi Biotec gentleMACS Dissociator (Gaithersburg, MD, USA). The small biopsy specimen from previously snap-frozen capsular tissue (0.5–1.0 mg) was transferred to specialized M Tubes (Miltenyi Biotec) with grinding units, along with 0.5 mL of Qiazol (Qiagen, Germantown, MD, USA). Following the manufacturer's instructions, the M Tube cap was closed tightly to the second click and then attached to the instrument, cap down. The preset program "RNA02" for 30 seconds was used for all frozen tissue. Samples were then transferred to microtubes for immediate RNA extraction by the standard phenol-chloroform method using Qiazol. The resulting RNA fraction was further purified using a Qiagen RNeasy mini spin column per the manufacturer's instructions. The eluate volume was 20 μL, and the resulting RNA concentration was determined using a spectrophotometer (NanoDrop 1000; Thermo Fisher Scientific, Waltham, MA, USA). The RNA quality was characterized by electrophoresis of 1.0 ng on a Bioanalyzer RNA chip (Agilent Instruments, Santa Clara, CA, USA). Assessment of the purity of all RNA preparations used in RNA sequencing showed RNA integrity numbers > 6.0.

Sequencing libraries were generated using 200 mg of mRNA via the TruSeq Stranded Total RNA high-throughput library preparation kit (Illumina, San Diego, CA, USA) according to the manufacturer's recommendations. The libraries were sequenced on an Illumina HiSeq 4000 system to 100 base pairs, single ended, and the sequencing depth was between 8.3 and 11.7 million reads per sample. Reads were aligned to the human genome (hg19) using the RNA-seq Unified Mapper (RUM) pipeline,[18] which also produced read-count quantifications of transcript expression. Differential expression was performed using EdgeR.[38] The resulting *P* values were converted to false discovery rates (FDRs) using

the R function “p.adjust” (R Foundation for Statistical Computing, Vienna, Austria). The RNA-sequencing analysis was carried out at the Generation Sequencing Core (Perelman School of Medicine, Philadelphia, PA, USA; ngsc.med.upenn.edu). The data are available at the National Center for Biotechnology Information Gene Expression Omnibus (NCBI GEO).

Data analysis and statistics

The functional and enrichment analysis of genes in idiopathic and diabetic AC patients was carried out using the Database for Annotation, Visualization and Integrated Discovery (DAVID)[24,25] with Gene Ontology (GO) annotations,[4,17] using Benjamini-adjusted *P* values to determine the significance of enriched biological pathways. RNA expression levels in patients with idiopathic AC were compared with those in nondiabetic patients without AC. Similarly, genetic expression levels in diabetic patients with AC were compared with those in diabetic patients without AC. Only genes with significantly different expression in idiopathic or diabetic AC patients, defined as having an FDR < 5%, were included for further analysis (Fig. 1).

Significant, differentially expressed genes in idiopathic AC were compared with the RNA expression profile in the diabetic AC cohort, and genes that differed by a fold change (FC) of at least ±2 were classified as specific to idiopathic AC. Significant genes in idiopathic AC patients that were within ±2 FC in the diabetic AC cohort, with an FDR < 50%, were classified as having similar expression in both subtypes of AC. The same process was repeated for differentially expressed genes in diabetic AC patients to classify genes specific to diabetic AC and genes with similar expression in both subtypes of AC.

Results

Histology

To confirm the location and examine differences in histopathologic findings, small portions of the rotator interval joint capsule taken for RNA preparation were taken for routine histologic analysis. Visualized with hematoxylin-eosin staining as well as safranin O with fast green counterstaining, the tissues were imaged and the tissue and cellular characteristics were evaluated (Fig. 2). All specimens showed characteristic dense layers of fibrous connective tissue with no marked differences in tissue architecture or cellularity except for diabetic AC patients’ samples, in which there were larger infiltrations of inflammatory cells.

RNA sequencing

RNA-sequencing results of shoulder capsular tissue demonstrated that 286 genes were significantly (FDR < 5%) differentially expressed in idiopathic AC as compared with 61 genes in diabetic AC when compared with nondiabetic controls and diabetic controls (Fig. 1). After removal of sex chromosome–linked genes from the data set, the expression levels of genes significant to idiopathic AC and diabetic AC were compared (Fig. 3). Most differentially expressed genes were upregulated in idiopathic AC (n = 251) and diabetic AC (n = 39). Of these upregulated genes, 92 of 251 were specific to idiopathic AC and 15 of 39 were specific to diabetic AC. Moreover, 54 of the upregulated genes had a similar FC in

both cohorts, and 8 of these genes were significantly upregulated in both subtypes of AC: *MMP9*, *MMP13*, *DGKI*, *KCNJ6*, *ADAM12*, *H19*, *POSTN*, and *ACAN*. In contrast, there were smaller numbers of significantly downregulated genes in idiopathic AC (n = 35) and diabetic AC (n = 12). Of these downregulated genes, 7 of 35 were specific to idiopathic AC and 2 of 12 were specific to diabetic AC. Furthermore, only 2 downregulated genes had a similar FC in both cohorts (*CSN1S1* and *TRIM14*), but neither was significantly downregulated in both subtypes of AC.

There was minimal change in RNA expression when comparing diabetic and nondiabetic patients without AC. Specifically, comparison of nonpathologic shoulder tissue demonstrated that only 3 genes were significantly differentially expressed. In contrast, diabetic and nondiabetic patients with AC had a greater change in RNA expression. On comparison of pathologic shoulder capsular tissue, 66 genes were differentially expressed between diabetic and nondiabetic patients (Fig. 3).

Gene enrichment

Enriched biological pathways identified in diabetic AC were related to hormonal and transcriptional changes, including cellular response to corticotropin-releasing factor (CRF) stimulus, steroid hormone–mediated signaling pathway, response to glucocorticoid, response to insulin, intracellular receptor signaling pathway, and positive regulation of transcription from RNA polymerase (Pol) II promoter (Fig. 4). Other pathways in diabetic AC were relevant to cell differentiation and regulation, including skeletal muscle cell differentiation, fat cell differentiation, cellular response to fibroblast growth factor stimulus, and inflammatory response. The enriched biological pathways in idiopathic AC involved muscle cells and extracellular matrix and included pathways such as muscle filament sliding, muscle contraction, and extracellular matrix organization. Three pathways were common in both subtypes of AC: extracellular matrix disassembly, skeletal system development, and cell adhesion.

The most significantly enriched biological pathways were compared in AC (Figs. 5 and 6) and found to be distinct. The pathways with the higher-fold enrichment in idiopathic AC were associated predominantly with structural element pathways, whereas the pathways with higher-fold enrichment in diabetic AC included fewer structural pathways and demonstrated greater association with hormonal and cell signaling pathways. In idiopathic AC, the highest number of significantly upregulated genes expressed belonged to the muscle contraction pathway (n = 26). In diabetic AC, the highest number of significantly upregulated genes expressed belonged to the transcription from the Pol II promoter pathway (n = 10). When only the unique genes were analyzed, the pathway with the highest-fold enrichment in idiopathic AC was skeletal muscle thin filament assembly, and in diabetic AC, the pathway with the highest-fold enrichment was cellular response to CRF stimulus. The related genes and biological pathways for idiopathic AC and diabetic AC are shown in Tables I and II and Supplementary Tables SII and S3.

Discussion

The currently accepted paradigm is that there is a presumed inciting stimulus for the development of AC that leads to differential expression of genes in both diabetic and normoglycemic cohorts. On comparison of nondiabetic and diabetic shoulder capsule specimens from patients without AC, only 3 genes were differentially expressed. In contrast, 66 distinctly different genes were expressed when nondiabetic and diabetic samples from patients with AC were compared. Thus, the presence of diabetes alone does not fully explain the transcriptomic differences in shoulder capsular tissue seen in AC. Future studies may reveal what the possible inciting factors are for the development of AC and its subsequent unique gene expression. Triggering elements could be physical and epigenetic factors including subtle trauma, surges in blood glucose levels, autoimmune factors, stress, or even a mild infectious agent.

In idiopathic AC, our analysis found that the collagen family of genes was involved in numerous pathways, including extracellular matrix organization, collagen catabolic process, collagen fibril organization, and cell adhesion.[44] Other relevant genes of interest found in idiopathic AC included the myosin, troponin, and actin family. In diabetic AC, the *NR4A1*, *NR4A2*, and *NR4A3* genes were represented in a number of the enriched pathways, including cellular response to CRF stimulus, fat cell differentiation, and positive regulation of transcription from RNA Pol II promoter.[42,54,55] The early growth response (EGR) family of transcription factors, including *EGR1*, *EGR2*, and *EGR3*, was also involved in a number of enriched biological pathways, including skeletal muscle cell differentiation and fat cell differentiation.[7] It appears, then, that idiopathic AC generally manifests with increased expression of genes related to muscle or collagen metabolism whereas diabetic AC is characterized by a panoply of DNA transcriptional (*POL2*), cell division regulation (*NR4A*), and hormonal (CRF) alterations.

When we more closely examine similarities and differences in the idiopathic AC and diabetic AC cohorts, some generalizations may be made. Both groups manifested upregulation of extracellular matrix disassembly, cell adhesion, and skeletal system development, suggesting perhaps an attempt at increased tissue remodeling.[23] For the idiopathic AC cohort, many genes were relevant to muscle tissue and included muscle filament sliding, muscle contraction, sarcomere organization, muscle organ development, skeletal muscle thin filament assembly, collagen fibril and extracellular matrix organization, and myofibril assembly, consistent with the anabolic and increased connective tissue production seen.[22] However, in diabetic AC, many upregulated genes are related to hormonal and transcriptional changes, including cellular response to CRF stimulus, steroid hormone–mediated signaling pathway, response to glucocorticoid, response to insulin, intracellular receptor signaling pathway, and positive regulation of transcription from RNA Pol II promoter.[48]

Numerous pathways have been implicated in the pathogenesis of AC, but the underlying pathophysiology remains unclear. Upregulation of inflammatory and fibroproliferative pathways has long been held as fundamental to the process; however, angiogenesis, neurogenesis, and cartilage differentiation have also been implicated. The specific pathways

responsible for AC have been investigated in previous studies, with heterogeneous results, likely owing to variation in timing and tissue location, as well as inclusion of patients with varying risk factors.[3,12,33] Moreover, studies exploring the pathophysiology of AC have often failed to make a distinction between patients with diabetes and those without diabetes. Other studies that did made a distinction have included only either idiopathic AC patients or diabetic AC patients without comparing the 2 cohorts. It is important to note that in this study, we report findings that confirm the distinct pathophysiology and synergistic effect of diabetes on AC in patients as compared with those without known risk factors for the disease.

In a previous study, a comparison of gene expression and translation among 12 patients with AC, including 2 patients with diabetes, Hagiwara et al[19] found various differentially regulated genes, 11 of which were consistent with those in our study. These genes included *ACAN*, *COL1A1*, and *SPARC*, which were upregulated in both idiopathic AC and diabetic AC; *EGR1*, *EGR2*, *NR4A1*, *ATF3*, *LIPG*, and *C1QTNF6*, which were upregulated only in diabetic AC; and *GPX3* and *SEMA3E*, which were only downregulated in idiopathic AC.[19] On further proteomic analysis of AC, which excluded diabetic AC patients, Hagiwara et al[20] found several upregulated pathways that were consistent with our findings identified in idiopathic AC but not in diabetic AC. These pathways included sarcomere organization, collagen catabolic process, collagen fibril organization, extracellular matrix organization, cell adhesion, and cell-matrix adhesion.[20] These data further validate our findings that tissue repair, collagen metabolism, cell-cell adhesion, and cell-matrix adhesion are important processes for idiopathic AC whereas diabetic AC has a separate underlying pathophysiology.

In a recent genome-wide expression study using RNA sequencing of 22 patients with AC, without the identification of diabetic status, 50 enriched pathways were identified.[28] Regarding these identified pathways, we also found that 6 of 29 biological process pathways were upregulated in our idiopathic AC group whereas 3 of 11 biological process categories were similarly upregulated in our diabetic AC group. Concordant pathways for idiopathic AC included collagen catabolic process, collagen fibril organization, extracellular matrix disassembly, and ventricular cardiac muscle tissue development, whereas concordant pathways for diabetic AC included extracellular matrix disassembly, skeletal muscle cell differentiation, and response to glucocorticoid.

Previous studies have described the upregulation of IL-6 and various other interleukins in shoulder capsule samples and joint fluid from patients with idiopathic AC.[1,35] Our results showed that IL-6 was similarly upregulated in the diabetic AC cohort, suggesting that there may be a common upstream inflammatory pathway in both subtypes of AC. Kim et al[32] also reported on the upregulation of intercellular adhesion molecule 1 (ICAM-1) in capsular tissue and synovial fluid from patients with AC. It is interesting to note that ICAM-1 was not directly upregulated in either subtype of AC in our study, but EGR, a transcription factor known to regulate the expression of ICAM-1, was upregulated in idiopathic AC.[16] In our study, *EGR1*, *EGR2*, *EGR3*, and *NR4A1* were all upregulated in diabetic AC but not idiopathic AC, and *ACAN* was upregulated in both diabetic AC and idiopathic AC. This finding suggests that *ACAN*, a cartilage-related gene, may play a similar chondrogenic role underlying some of the pathophysiology in both subtypes of

AC. In contrast, it is likely that the transcription factors comprising the EGR family, which have been implicated as key mediators for inflammation and fibrosis through transforming growth factor β regulation, play a more important role in diabetic AC.[7] These results point to the possibility of developing anti-EGR therapies targeted toward diabetic AC. Furthermore, *NR4A1*—an orphan nuclear receptor that has been implicated in various metabolic processes such as carbohydrate and lipid metabolism—has also recently been implicated as a key mediator of transforming growth factor β signaling and fibrosis.[42,54,55] As such, the nuclear orphan receptor (*NR4A*) superfamily presents a promising therapeutic opportunity in diabetes-induced AC and warrants further study.

Taken together, our findings identify distinct underlying pathophysiology between idiopathic AC and diabetic AC. Our findings in idiopathic AC patients are consistent with findings in the existing literature from studies of AC; however, our findings in diabetic patients are unique.[20] Furthermore, our data suggesting a distinct pathophysiology between idiopathic AC and diabetic AC are supported by the disparate outcomes observed clinically in diabetic patients with AC, including more frequent failure of conservative management and need for arthroscopic capsule release.[31,46] Despite the unique upstream pathophysiology, there are some common pathways regarding structural elements between both subtypes of AC, potentially explaining the similar initial clinical presentation of idiopathic AC and diabetic AC. In our study, we found that important extracellular matrix proteases *MMP1*, *MMP13*, *MMP16*, *MMP3*, and *MMP9* were upregulated in idiopathic AC and *MMP9* and *MMP13* were also upregulated in diabetic AC.

Diabetes mellitus has been known to affect numerous organ systems[34] and influence multiple hormonal pathways.[39] We have presented yet another way in which diabetes may alter CRF, insulin, and glucocorticoid transcription. Other pathways in diabetic AC identified in our study were relevant to cell differentiation and regulation, including skeletal muscle cell differentiation, fat cell differentiation, cellular response to fibroblast growth factor stimulus, and inflammatory response. Of note, the upregulation of fat cell differentiation in diabetic AC samples is of special interest and merits further study. It has been clearly established that diabetes mellitus is linked to obesity and increased dietary free fatty acid levels.[6] However, impaired fat cell differentiation has also been shown to be strongly associated with insulin resistance and diabetes mellitus.[26]

The more virulent clinical course of diabetic AC can be explained by the consequential protein and hormonal gene upregulation demonstrated in this work. Studies undertaking biological evaluation of AC should take great care in recognizing the different patient risk factors that may have an impact on underlying pathophysiology. The difference in underlying pathophysiology for idiopathic AC and diabetic AC suggests that medical treatment for frozen shoulder may be preferentially targeted to certain subtypes of AC. As such, there is biological justification for developing different treatment algorithms for diabetic patients and nondiabetic patients with frozen shoulder. With identification of the transcriptomic constitution of each disease entity, new and targeted treatment strategies can be developed.

Although sex differences were not the focus of this study, it is important to keep in mind that our study was performed in subject populations of both sexes, with the exception that diabetic AC shoulder capsule samples were only obtained from female patients. However, all significantly differentially expressed sex chromosome–specific genes were removed from the data set following RNA-sequencing analysis. Moreover, the enriched biological pathways identified by gene ontology (GO) analysis have not been found in previous studies comparing RNA-sequencing results between male and female patients. A previous study also found that sex did not have a major impact on gene expression levels from shoulder samples in patients with AC.[28]

Another limitation is that the mean hemoglobin A_{1c} level in diabetic patients was 8.7%, suggesting that many patients had uncontrolled diabetes. As such, the level of diabetic control preceding the collection of shoulder capsule specimens could have significantly varied between patients, thus affecting the results of this study. There was also variation in treatment history between patients with AC and those without AC. The majority of all patients with AC had received at least a single intra-articular steroid injection whereas only 2 patients without AC had received at least a single intra-articular injection, potentially affecting shoulder capsule gene expression results. However, this is unlikely to have affected the comparison of gene expression between diabetic AC and idiopathic AC as both diabetic and nondiabetic patients with AC were more likely to have received steroid injections compared with their respective control groups. Furthermore, the presence of metabolic syndrome or dyslipidemia was not evaluated in every subject. It is possible that the presence of dyslipidemia or metabolic syndrome may have been a confounding variable in this study, and further studies should evaluate the impact of dyslipidemia and metabolic syndrome on AC pathophysiology. Similarly, 3 nondiabetic patients were not screened for A_{1c} levels because they had no symptoms of diabetes or relevant risk factors, such as a family history or obesity. However, we cannot definitively exclude the possibility that these 3 patients did not have unrecognized diabetes, which also could have affected the results of this study.

Conclusion

This study has shown for the first time that transcriptome expression profiles demonstrate a fundamentally different underlying pathophysiology for diabetic AC and idiopathic AC. These findings suggest that these conditions are distinct clinical entities. In translational terms, the new genes expressed reveal potentially new therapeutic targets, and further characterization may lead to different treatment paradigms in these 2 clinical subsets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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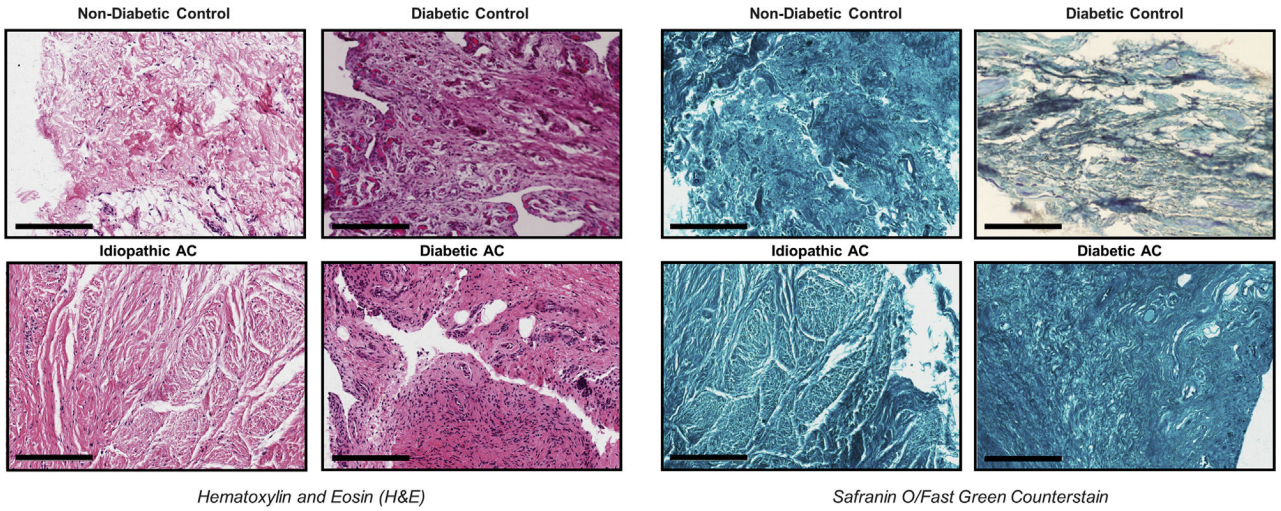

Figure 1.

Genetic expression differences in idiopathic and diabetic adhesive capsulitis patients compared with controls without AC. The RNA-sequencing results of gene expression differences in patients with adhesive capsulitis as compared with diabetic patients with adhesive capsulitis are shown. Patients with regular adhesive capsulitis were compared with normal control patients without adhesive capsulitis, and diabetic patients with adhesive capsulitis were compared with diabetic patients without adhesive capsulitis. *FDR*, false discovery rate; *M*, fold change.

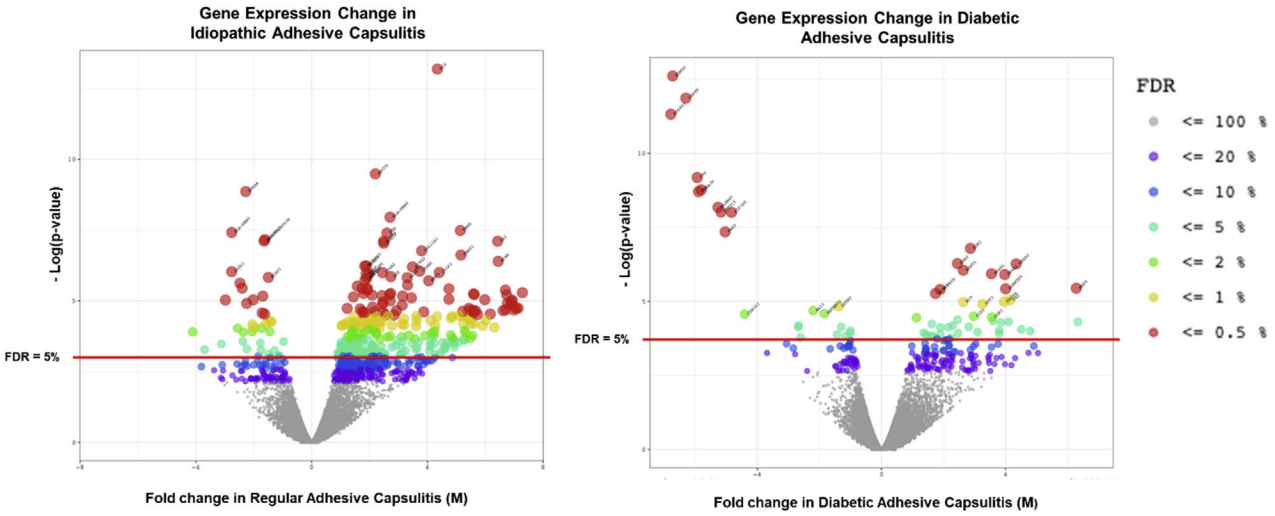

Figure 2.

Representative histologic sections of capsular tissue in idiopathic and diabetic adhesive capsulitis *(AC)* demonstrating tissue and cellular characteristics. Staining was performed with hematoxylin-eosin *(left panels)* and safranin O with fast green counterstaining *(right panels)*. *Scale bars* represent 200 μm.

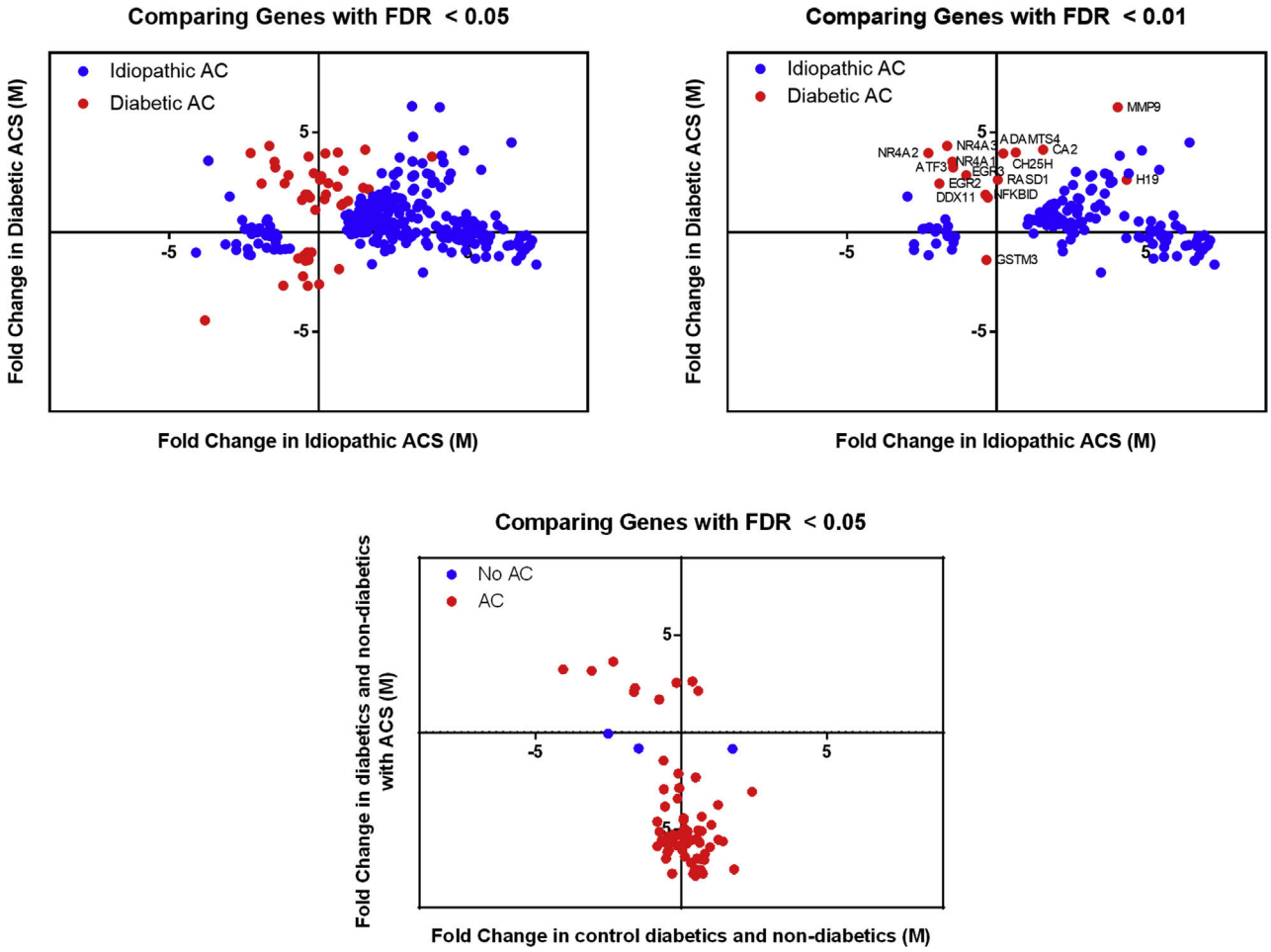

Figure 3.

Comparison of genetic expression levels across various cohorts. The fold change of genes that were significantly (false discovery rate *[FDR]* < 0.05) differentially expressed in idiopathic or diabetic adhesive capsulitis *(ACS/AC)* were compared across patient cohorts. Additionally, the fold change of significantly differentially expressed genes in patients with AC or without AC were compared across cohorts. *M*, fold change.

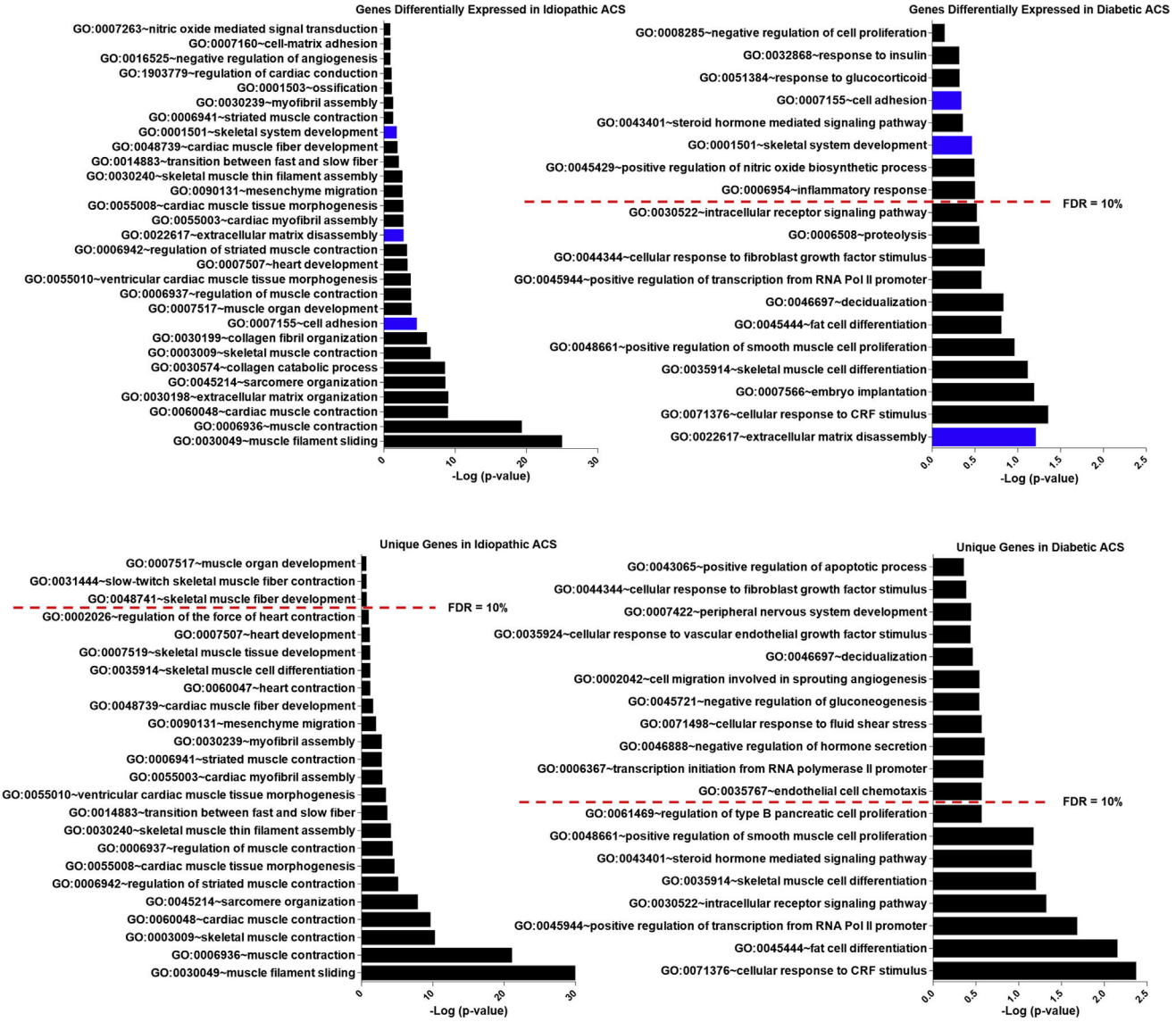

Figure 4.

Enriched biological pathways in adhesive capsulitis *(ACS)*: gene enrichment analysis of genes significantly associated with idiopathic and diabetic adhesive capsulitis. Biological pathways in *blue* were implicated in both idiopathic adhesive capsulitis and diabetic adhesive capsulitis. *GO*, Gene Ontology; *FDR*, false discovery rate; *Pol*, polymerase; *CRF*, corticotropin-releasing factor.

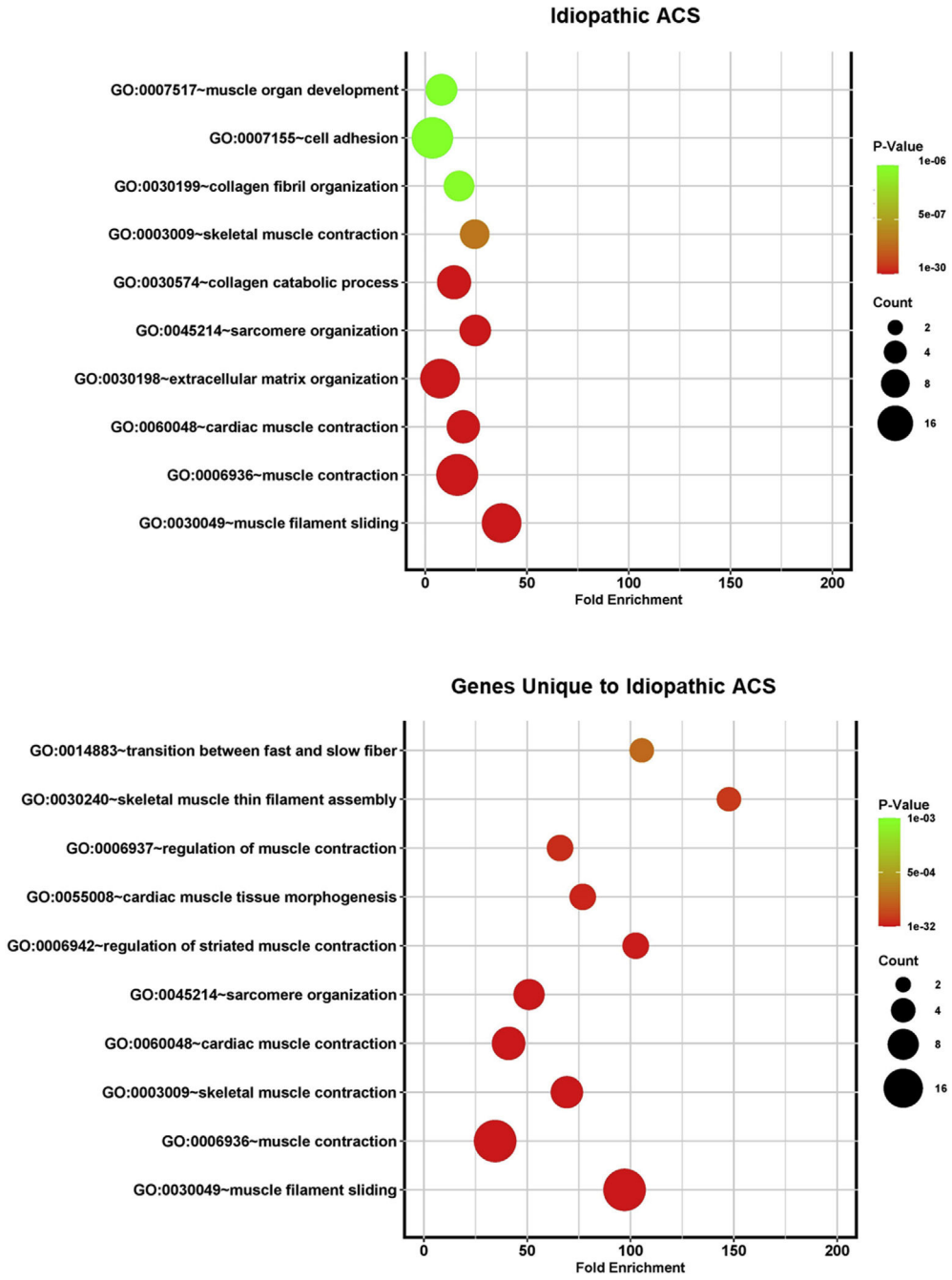

Figure 5.

Bubble plots demonstrating fold enrichment for the most statistically significant biological pathways identified by Gene Ontology *(GO)* analysis for idiopathic adhesive capsulitis *(ACS)*.

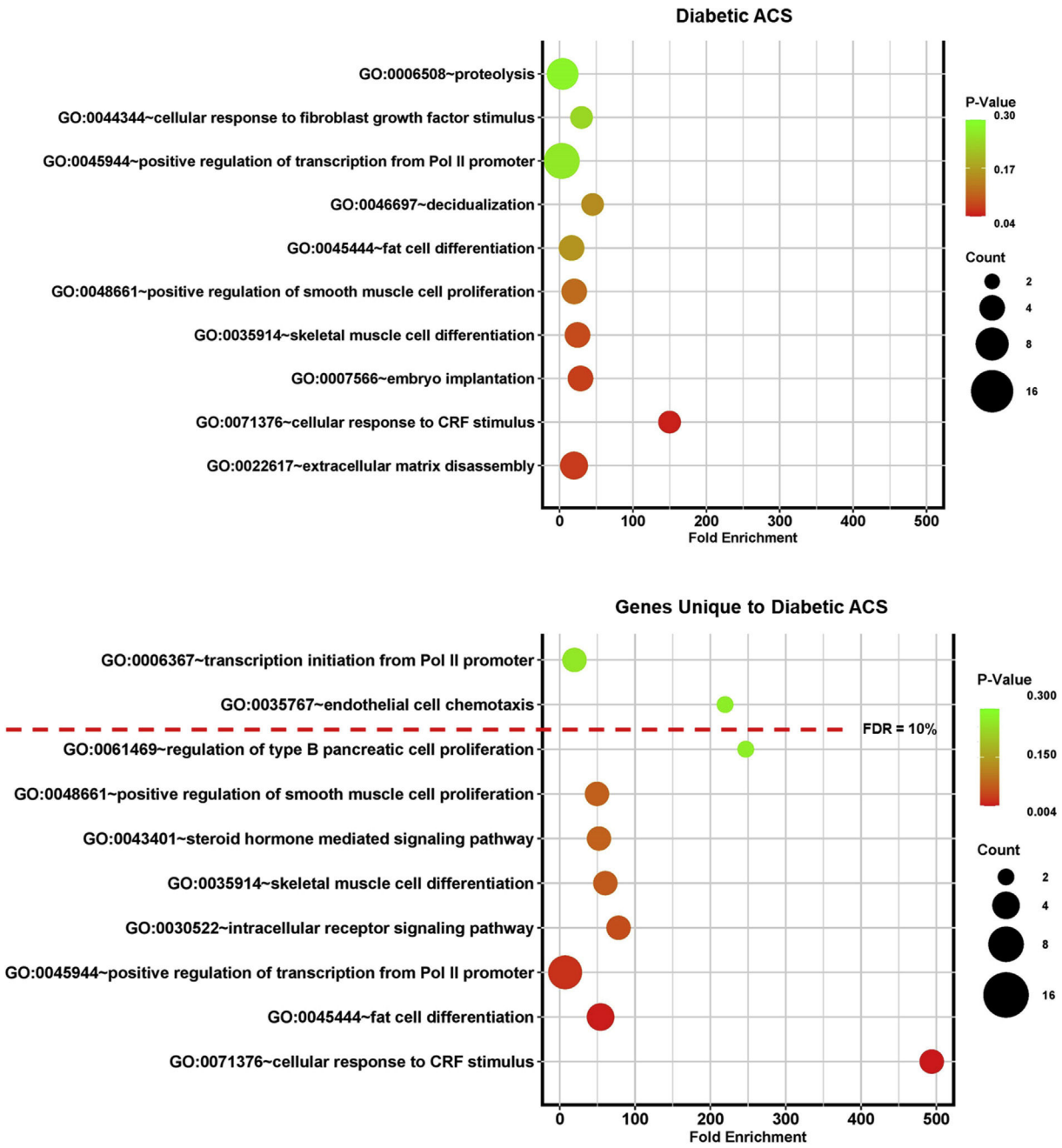

Figure 6.

Bubble plots demonstrating fold enrichment for the most statistically significant biological pathways identified by Gene Ontology *(GO)* analysis for diabetic adhesive capsulitis *(ACS)*. *Pol*, polymerase; *CRF*, corticotropin-releasing factor.

Table I

Differentially expressed genes related to 10 most statistically significant biological pathways identified by GO analysis for idiopathic AC

Biological pathway	Related genes
GO:0030049—muscle filament sliding	*ACTA1, ACTC1, ACTN2, DES, MYBPC2, MYBPC1, MYH2, MYH7, MYL1, MYL2, MYL3, NEB, TTN, TCAP, TPM1, TPM2, TNNC1, TNNC2, TNNI1, TNNI2, TNNT1, TNNT3*
GO:0006936—muscle contraction	*ACTA1, ACTA2, ACTG2, CACNA1S, CKMT2, DES, DYSF, HRC, LMOD2, LMOD3, MYOM1, MYOM2, MYBPC2, MYH1, MYH2, MYH7, MYL1, MYLK, MYLPF, MYOT, TTN, TRDN, TRIM63, TPM1, TPM2, VIPR1*
GO:0060048—cardiac muscle contraction	*ACTC1, MYH7, MYL1, MYL2, MYL3, RYR2, SCN4B, TTN, TCAP, TPM1, TNNC1, TNNI1, TNNI2*
GO:0030198—extracellular matrix organization	*ACAN, COL1A1, COL1A2, COL3A1, COL5A1, COL5A2, COL5A3, COL11A1, COL16A1, EMILIN1, ELN, ITGA2, ITGA7, ITGA8, LOX, NDNF, NID1, NID2, OLFML2B, POSTN, SPARC*
GO:0045214—sarcomere organization	*LDB3, ACTN2, ANKRD1, CASQ1, FHOD3, KLHL41, LMOD2, OBSCN, TTN, TCAP, TPM1*
GO:0030574—collagen catabolic process	*COL1A1, COL1A2, COL3A1, COL5A1, COL5A2, COL5A3, COL6A5, COL11A1. COL18A1, MMP1, MMP13, MMP16, MMP3, MMP9*
GO:0003009—skeletal muscle contraction	*STAC3, MYH7, TCAP, TNNC1, TNNC2, TNNI1, TNNI2, TNNT1, TNNT3*
GO:0030199—collagen fibril organization	*ACAN, COL1A1, COL1A2, COL3A1, COL5A1, COL5A2, COL5A3, COL11A1, LOX, SERPINH1*
GO:0007155—cell adhesion	*ADAM12, NUAK1, THY1, ACTN2, ACAN, CDH11, COL1A1, COL5A1, COL6A5, COL16A1, COL18A1, EMILIN1, ITGA2, ITGA7, ITGA8, LRRN2, MYBPC2, MYBPC1, NCAM1, NID2, POSTN, PCDH17, RELN, SPON1, STAB2*
GO:0007517—muscle organ development	*CHODL, FHL3, HBEGF, ITGA7, MAPK12, MKX, MURC, NEB, SMTN, TAGLN, UNC45B*

GO, Gene Ontology; *AC*, adhesive capsulitis.

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Table II

Differentially expressed genes related to 10 most statistically significant biological pathways identified by GO analysis for diabetic AC

Biological pathway	Related genes
GO:0022617—extracellular matrix disassembly	*ADAMTS4, ACAN, MMP13, MMP9, SPP1*
GO:0071376—cellular response to CRF stimulus	*NR4A1, NR4A2, NR4A3*
GO:0007566—embryo implantation	*LIF, MMP9, PTGS2, SPP1*
GO:0035914—skeletal muscle cell differentiation	*ATF3, EGR1, EGR2, NR4A1*
GO:0048661—positive regulation of smooth muscle cell proLiferation	*AIF1, IL6, NR4A3, PTGS2*
GO:0045444—fat ceU differentiation	*EGR2, NR4A1, NR4A2, NR4A3*
GO:0046697—decidualization	*LIF, PTGS2, SPP1*
GO:0045944—positive regulation of transcription from RNA Pol II promoter	*FOSL2, ATF3, CSRNP1, EGR1, EGR2, IL6, LIF, NR4A1, NR4A2, NR4A3*
GO:0044344—cellular response to fibroblast growth factor stimulus	*EGR3, NR4A1, POSTN*
GO:0006508—proteolysis	*ADAM12, ADAMTS1, ADAMTS4, ACAN, C1QA, MMP13, MMP9*

GO, Gene Ontology; *AC*, adhesive capsulitis; *CRF*, corticotropin-releasing factor; *Pol*, polymerase.

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