J Shoulder Elbow Surg (2018) ■■, ■■–■■

ELSEVIER

Journal of Shoulder and Elbow Surgery

www.elsevier.com/locate/ymse

ORIGINAL ARTICLE

Comparative proteome analysis of the capsule from patients with frozen shoulder

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Background: The etiology of frozen shoulder (FS) is unclear. Accordingly, this study used a label-free quantitative shotgun proteomic approach to elucidate the pathogenesis of FS based on protein expression levels.

Methods: Tissue samples from the rotator interval (RI), middle glenohumeral ligament (MGHL), and anterior-inferior glenohumeral ligament (IGHL) were collected from 12 FSs with severe stiffness and 7 shoulders with a rotator cuff tear (RCT) as controls. Protein mixtures were digested and analyzed by nano-liquid chromatography/electrospray ionization–tandem mass spectrometry. Relative protein expression levels were calculated by the signal intensity of identified peptide ions on mass spectra. Differentially expressed proteins between FS and RCT samples were evaluated by a gene enrichment analysis using Gene Ontology and Kyoto Encyclopedia of Genes and Genomes.

Results: We identified 1594 proteins, 1358 of which were expressed in all 6 tissue groups. We detected more upregulated proteins in the upper (RI and MGHL) FS groups and the lower (IGHL) RCT group than in the comparative groups, respectively. Various proteins with functions in tissue repair, collagen metabolism and fibrillation, cell–cell and cell–matrix adhesion, blood coagulation, and the immune response were expressed more highly in the RI and MGHL FS groups than in the RCT group. Proteins with functions in phagocytosis, glutathione metabolism, retinoid metabolism, and cholesterol metabolism were expressed more highly in the IGHL RCT group than in the FS group.

Conclusions: The pathophysiology of FS differs between the upper and lower parts of the joint capsule. Different treatment strategies for FS may be appropriate, depending on the location.

Level of evidence: Basic Science Study; Molecular and Cell Biology

The Tohoku University School of Medicine Institutional Review Board approved the study protocol (approval number, 2015-1-483).

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https://doi.org/10.1016/j.jse.2018.03.010

Keywords: Frozen shoulder; quantitative proteome analysis; adhesive capsulitis; joint capsule; ligament; pathogenesis

Frozen shoulder (FS) is a common disease characterized by a decreased range of motion (ROM) and severe pain. In the case of persistent ROM restriction after appropriate conservative treatments, arthroscopic capsular release is a common method to regain ROM.[16] This indicates that changes in the joint capsule are among the main pathologies for the ROM restriction in FS.

Inflammation and fibrosis are potentially important in the pathogenesis of FS.[4,30] Although collagen density and stiffness in the joint capsule increase with the upregulation of various growth factors and cytokines,[13,24,37] proteoglycans also seem to play an important role in the development of FS.[13,25]

Shotgun proteome analyses by nano-liquid chromatography/electrospray ionization–tandem mass spectrometry can be used to elucidate the mechanisms underlying diseases. Many biomarkers have been identified using a proteomic approach, some of which have been used for diagnostic and therapeutic applications.[48] Recently, transcriptome analyses using next-generation sequencing have also been widely used because global messenger RNA expression levels can be acquired from trace amounts of samples.[10,19,38] However, messenger RNA and protein expression levels are weakly correlated in various species[26,41]; accordingly, an analysis of associations between global protein expression levels and biological phenomena is important. This study used shotgun proteome analysis and gene enrichment analysis of FS clinical specimens to elucidate the pathogenesis of FS based on protein expression levels.

Materials and methods

Patients and tissue collection

This was a retrospective case-control study of specimens from patients with FS or rotator cuff tear (RCT). All study participants provided informed consent. Inclusion criteria for FS were (1) a history of at least 1 month of pain and stiffness, (2) restriction of passive glenohumeral motion of 100° of forward flexion or less, 20° of external rotation or less, and internal rotation (fifth lumbar vertebra or lower), and (3) normal radiologic appearance.[35] All patients had a routine radiographic evaluation of anteroposterior views in internal rotation and external rotation, outlet views of bilateral shoulders, and magnetic resonance imaging.

The study excluded patients with glenohumeral osteoarthritis, calcific tendinitis, superiorly migrated humeral head, and osteonecrosis of the humeral head.[2] Patients with diabetes mellitus, other systemic metabolic diseases, and traumatic events were excluded.

Arthroscopic capsular release was performed for 12 patients with FS whose condition had failed to improve or had deteriorated after 6 months of intensive conservative treatment, indicating the frozen phase.

As a control group, 7 patients with RCT without severe limitation in ROM (≥140° of forward flexion and ≥40° of external rotation) or traumatic events were selected (Table I). Patients without systemic metabolic disorders, including diabetes mellitus, were included.

Biopsy materials from the rotator interval (RI), middle glenohumeral ligament (MGHL), and anterior-inferior glenohumeral ligament (IGHL) were obtained during surgery.[12,13] To avoid the influence of the synovium, the joint capsule itself was collected after shaving it in a case with proliferation.

The average ages of the FS (3 men and 9 women) and RCT (5 men and 2 women) groups were 56.3 years (range, 25-74; standard deviation, 13.2 years) and 63 years (range, 46-79; standard deviation, 12.9 years), respectively. The difference in age between the 2 groups was not significant ($P = .29$ by Student t test). The evaluation excluded 4 RI and 2 IGHL samples to insufficient protein extractions (Table I).

Sample preparation

Approximately 20 mg of each shoulder tissue sample was placed in 300 μL of 100 mmol/L triethylammonium bicarbonate buffer (pH 8.5) containing 12 mmol/L sodium deoxycholate, 12 mmol/L sodium *N*-dodecanoyl sarcosinate, and 1% protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA) and broken using the BiomasherII (Nippi Protein Engineering Office, Tokyo, Japan) and sonication for 30 minutes. The mixtures were centrifuged at 15,000*g* for 3 minutes, and the supernatants were diluted to obtain a total protein concen-

Table I List of patients and tissues

Diagnosis	Age (yr)	Sex	Left/right	Site		
				RI	MGHL	IGHL
RCT	46	Male	Right	●	●	●
RCT	51	Male	Right	●	●	●
RCT	53	Female	Right	●	●	●
RCT	66	Male	Left	●	●	●
RCT	71	Female	Right	●	●	●
RCT	75	Male	Right	●	●	●
RCT	79	Male	Right	●	●	
FS	25	Female	Left		●	●
FS	44	Female	Right		●	●
FS	49	Female	Right	●	●	●
FS	51	Female	Right	●	●	●
FS	53	Male	Right		●	●
FS	58	Male	Right		●	●
FS	59	Female	Right	●	●	●
FS	63	Female	Left	●	●	
FS	64	Male	Left	●	●	●
FS	66	Female	Right	●	●	●
FS	69	Female	Right	●	●	●
FS	74	Female	Left	●	●	●

RI, rotator interval; *MGHL*, middle glenohumeral ligament; *IGHL*, anterior-inferior glenohumeral ligament; *RCT*, rotator cuff tear; *FS*, frozen shoulder.