Proteomic Expression Profile in Human Temporomandibular Joint Dysfunction

Andrea Duarte Doetzer 1*, Roberto Hirochi Herai 1, Marilia Afonso Rabelo Buzalaf 2 and Paula Cristina Trevilatto 1

Abstract: Temporomandibular joint dysfunction (TMD) is a multifactorial condition that impairs human's health and quality of life. Its etiology is still a challenge due to its complex development and the great number of different conditions it comprises. One of the most common forms of TMD is anterior disc displacement without reduction (DDWoR) and other TMDs with distinct origins are condylar hyperplasia (CH) and mandibular dislocation (MD). Thus, the aim of this study is to identify the protein expression profile of synovial fluid and the temporomandibular joint disc of patients diagnosed with DDWoR, CH and MD. Synovial fluid and a fraction of the temporomandibular joint disc were collected from nine patients diagnosed with DDWoR (n = 3), CH (n = 4) and MD (n = 2). Samples were subjected to label-free nLC-MS/MS for proteomic data extraction, and then bioinformatics analysis were conducted for protein identification and functional annotation. The three TMD conditions showed different protein expression profiles, and novel proteins were identified in both synovial fluid and disc sample. TMD is a complex condition and the identification of the proteins expressed in the three different types of TMD may contribute to a better comprehension of how each pathology develops and evolves, benefitting the patient with a focus–target treatment.

Keywords: temporomandibular joint; protein expression; temporomandibular joint dysfunction

1. Introduction

Temporomandibular dysfunction (TMD) is a disorder of the masticatory system and it is characterized by pain, loss of function of one or both articulations, and impairment of the masticatory system. TMD impacts not only jaw function, but the life quality of affected patients, increasing their treatment costs and work absence [1]. According to the National Institute of Health [2], TMD management in the USA costs approximately 4 billion dollars per year. A diagnostic protocol developed for research named Research Diagnostic Criteria for TMD (RDC/TMD), classifies TMD as myalgia, arthralgia, condylar pathologies, disc displacement, osteoarthrosis, osteoarthritis, degenerative joint disease and subluxation [3]. TMD has a multifactorial etiology, the most common being trauma, psychological alterations, hormone, inflammatory diseases, parafunction, and genetics [1,4]. TMD usually requires a panorex, and depending on the TMD type, magnetic resonance imaging, scintigraphy and tomography, besides a thorough clinical evaluation [5,6].

Depending on the TMD type, it can be classified as condylar hyperplasia (CH), disc displacement without reduction (DDWoR) and mandibular dislocation (MD). DDWoR is the most common TMD disorder [7], and along with CH, its etiology’s understanding is still unclear. MD is a condition that is probably caused by physical alterations [8], and since it is less likely to have hormone contribution, it is a good TMD condition to compare the results with the other pathologies. DDWoR is caused by an abnormal positional association between the disc and the condyle, where the disc is permanently anteriorly displaced...
in relation to the condyle, causing limited range of mouth opening, pain and may lead to temporomandibular joint (TMJ) degeneration [9]. Disc displacement corresponds to 41% of TMD intra-articular disorders [7], and it is considered a multifactorial disease, with overlapping conditions contributing to its modulation including stress, parafunction, behavioral pattern, emotional status, and genetic background [3]. Among its different types of treatment, clinical handling is firstly employed (splint therapy, medication, physiotherapy) and when unsuccessful, surgery is indicated [6,10]. MD is an involuntary forward movement of the condyle beyond the articular eminence, mostly associated with trauma or excessive mouth opening, impairing its essential functions (speaking, chewing), and it accounts for 3% of all documented dislocations [11]. It usually needs mechanical manipulation to return to its normal position, and recurrent dislocations require surgical treatment [8]. Between these TMD types, CH is the rarest pathology that manifests a head condyle overgrowth, causing facial asymmetry, deformity, malocclusion and sometimes pain and dysfunction [12]. It is a self-limiting condition, more prevalent in female teenagers, but it usually requires surgical treatment to limit facial asymmetry progression and condyle continuous elongation [13]. Studies suggest it has a genetic involvement on its development, but its main etiology is still poorly understood [14].

Despite the etiological differences between CH, DDWoR and MD, current studies have limited understanding of the molecular variations that differentiates these TMD diseases. Condylar hyperplasia, mandibular dislocation and disc displacement have been the aim of many studies, due to their difficulty in targeting the proper treatment to each disease [9]. The employment of specific treatment, which may be improved with the unveiling of its specific etiology factors, will allow us to diminish treatment time and costs.

At the proteomic level, current studies focus only on individual mandibular dysfunctions, without comparing different TMD types to show the proteomic variability that could drive novel biomarkers as targets for disease diagnostic and treatment [15,16]. Proteomic analysis is a gold standard approach to analyze all identifiable proteins in a certain tissue, investigating its abundance, variety of proteoforms, and their stable or transient protein–protein interactions. This approach is especially beneficial in the clinical setting when studying proteins involved in different pathologies [17]. To date, there are very few studies investigating human TMD samples through proteomic output, and these studies analyzed only synovial fluid, focusing on specific target proteins [15,16]. Therefore, analyzing all proteins present in the synovial fluid and disc sample of different types of TMD may potentially lead TMD treatments towards a new reality.

In this research, a high throughput proteomic investigation of the three TMD pathologies CH, DDWoR and MD, was performed. Using state-of-the-art sample extraction procedures, biological samples of synovial fluid and TMJ discs were collected from distinct patients diagnosed with these conditions. The samples were processed, subjected to protein extraction and mass spectrometry proteomic identification. Generated proteomic data were analyzed using bioinformatics methods, and a per-sample protein identification and annotation were performed. The clinical phenotypes were then used to correlate the proteomic profile of each TMD condition.

2. Materials and Methods

2.1. Sample Selection

The sample was composed of 9 disc and synovial fluid specimens from female patients, with a mean age of 31.22 years (18–52). The patients presented different TMJ conditions, with three samples being composed of TMJ displaced disc without reduction (n = 3), two mandibular dislocation (n = 2) and four patients with condylar hyperplasia (n = 4) (Table 1). The specimens were collected from patients treated at the Evangelic University Hospital of Curitiba, Brazil. The study was approved by the Ethical Committee on Research at Pontifical Catholic University of Paraná, Brazil, according to Resolution 196/96 of the National Health Council and approved on 6 May of 2016 under registration number 1.863.521.
Table 1. Baseline characteristics of the sample, showing age and pathology of each female patient.

| Number | Age | Diagnostic                                |
|--------|-----|-------------------------------------------|
| 1      | 18  | Condylar Hyperplasia                      |
| 2      | 20  | Condylar Hyperplasia                      |
| 3      | 38  | Mandibular Dislocation                    |
| 4      | 38  | Mandibular Dislocation                    |
| 5      | 36  | Condylar Hyperplasia                      |
| 6      | 29  | Condylar Hyperplasia                      |
| 7      | 25  | Disc Displacement Without Reduction       |
| 8      | 25  | Disc Displacement Without Reduction       |
| 9      | 52  | Disc Displacement Without Reduction       |

Subjects did not present any of the following criteria: use of orthodontic appliances; chronic usage of anti-inflammatory drugs; history of diabetes, hepatitis, HIV infection; immunosuppressive chemotherapy; history of any disease known to compromise immune function; pregnancy or lactation; major jaw trauma; previous TMJ surgery; and previous steroid injection in the TMJ.

Subjects answered a personal medical history questionnaire and signed a consent form after being advised of the nature of the study. All patients were clinically examined by one experienced oral and maxillofacial surgeon. The clinical examination consisted of palpating the TMJ region, analyzing the occurrence of painful or limitation/excessiveness of mouth opening/closing, and the observation of facial asymmetry. Regarding complementary exams, all patients had a panorex and patients with disc displacement were submitted to a magnetic resonance image. The patients who were considered to be affected with disc displacement were treated surgically when they presented painful clinical signs of disc displacement after unsuccessful non-surgical treatment for at least 6 months [18].

Patients presenting pain related only to muscular spasms were not included in this research. Patients with condylar hyperplasia were diagnosed through clinical evaluation, panorex and when presenting a positive condylar growth in scintilography, a high condylectomy was indicated and performed [19]. Patients with recidivist mandibular dislocation (more than four episodes in six months) were treated with eminectomy [8].

2.2. Sample Acquisition

During access to the TMJ to perform the needed surgery [20], a 21-gauge needle was inserted into the upper TMJ space, then 1 mL of saline was injected into the joint space, which was aspirated thereafter by a second adapted syringe. This procedure was repeated five times to obtain a synovial fluid sample as described previously by Alstergren [21]. For each type of surgery performed, TMJ disc recontouring and repositioning was needed [16], therefore, first the displaced disc was freed, repositioned and sutured to the latero-posterior side of the condyle with a Mitek bone-cleat. The suture was then placed between the posterior and intermediate bands, and recontouring the thickened disk with a scalpel was necessary (this posterior debrided cartilage constituted the disc sample). Synovial fluid was spun down at 300 × g to remove debris, and stored at −80 °C until use or analysis, and the disc samples rinsed in phosphate-buffered saline (PBS), and either snap frozen in liquid nitrogen and stored at −80 °C.

2.3. Proteomic Analysis

The microcentrifuge tubes containing the synovial fluid and TMJ discs were removed from the −80 °C freezer, and after defrosting, the discs were cut into small pieces with the aid of sterile scissors, centrifuged, and the supernatants were collected and pooled according to each pathology group. The preparation of the samples for proteomic analysis was carried out as previously reported [22]. The analysis of the tryptic peptides was performed in the nanoACQUITY UPLC system (Waters, Milford, CT, USA) coupled to the Xevo Q-TOF G2 mass spectrometer (MS) (Waters, Milford, CT, USA). For this purpose,
the UPLC nanoACQUITY system was equipped with a column of type HSS T3 (Acquity UPLC HSS T3 column 75 mm × 150 mm; 1.8 µm, Waters), previously balanced with 7% of the mobile phase B (100% ACN + 0.1% formic acid). The peptides were separated through a linear gradient of 7%–85% of the mobile phase B over 70 min with a flow of 0.35 µL/min and the column temperature maintained at 45 °C. The MS was operated in positive ion mode, with a 75 min data acquisition time. The obtained data were processed using ProteinLynx GlobalServer (PLGS) version 3.03 (Waters, Millford, CT, USA). Protein identification was obtained using the ion counting algorithm incorporated into the software. The collected data were searched in the database of the species Homo sapiens downloaded from the catalog of the UniProt [23] in September of 2020. The identified proteins for the groups DDWoR, MD, and CH of synovial fluid and TMJ disc were classified and attributed by biological function, origin, and molecular interaction with the program Genemania [24]. The overlapping proteins between the groups were clustered by using an automatic Venn diagram generator.

3. Results

In this qualitative study, our aim was to explore, for the first time, a comparative analysis of the proteomic profile of three distinct TMD diseases. Although a statistical analysis was not performed, we were able to identify and describe the function of the proteins, including overlapping proteins between the investigated samples (DDWoR, MD, and CH and between both synovial fluid and disc samples).

In the synovial fluid samples, a total of 225 proteins (351 counting the repeated proteins in all groups) were successfully identified: 190 in the group DDWoR, 154 in the group MD and seven in the group CH. We also compared these three groups to identify shared or condition-specific proteins. We found 114 shared proteins between groups DDWoR and MD, and six proteins were shared by all groups (Table 2).

In the disc sample, 379 proteins were identified (697 counting the repeated proteins in all groups), with 235 proteins in group DDWoR, 196 in group MD and 266 in group CH. These three groups were also compared to identify shared or condition-specific proteins. There were nine shared proteins between groups DDWoR and MD, 28 shared proteins between groups DDWoR and CH, 17 shared proteins between groups MD and CH, and 132 shared proteins by all groups (Table 3).

Regarding the proteins in common in both synovial fluid and disc in the same sample groups, DDWoR presented two common proteins, MD presented three proteins, group CH had no protein in common, and the three groups together had six proteins in common (Table 4).

All synovial fluid and disc samples presented proteins involved in DNA repair, muscle and neural regeneration.

A selective pool of proteins was chosen to be studied according to the pathology group and protein function for synovial fluid and disc sample (Tables 5 and 6).

The synovial fluid sample presented the following proteins functions for each group (Table 5): the DDWoR group presented proteins involved in inflammatory process, apoptosis, hearing, interleukine-6 cascade, and protection against oxidative stress; the MD group showed proteins involved in inflammatory process, apoptosis, hearing, interleukine-6 cascade, protection against oxidative stress, and immune response; in the CH group, the expression of alcohol degradation protein (ADH1) was identified. The group comprising the pathologies DDWoR and MD were mainly involved in inflammatory process inhibition, bone resorption, chondrogenesis, bone and cartilage formation, osteoarthrosis, and neuropathic pain. No proteins were observed in the groups DDWoR and CH, and MD and CH. The proteins expressed in all three groups (DDWoR, MD and CH) were mainly implicated with muscle regeneration.
Table 2. Gene code and name of the proteins expressed in synovial fluid of all groups (disc displacement without reduction (DDWoR), mandibular dislocation (MD), condylar hyperplasia (CH)) and between the groups DDWoR and MD, DDWoR and CH, MD and CH and DDWoR, MD and CH.

| Protein Expressed in Each Group of TMJ Synovial Fluid Sample (n = 225) | DDWoR (n = 70) | MD (n = 34) | CH (n = 1) | DDWoR and MD (n = 114) | DDWoR and CH (n = 0) | MD and CH (n = 0) | DDWoR, MD and CH (n = 6) |
|---|---|---|---|---|---|---|---|
| Code | Name | Code | Name | Code | Name | Code | Name | X | X | Code | Name |
| A2M | Alpha-2-Macroglobulin | ACTR3B | Actin Related Protein 3B | ADH1 | Alcohol Dehydrogenase | ABI3BP | ABI Family Member 3 Binding Protein | ENO1 | Enolase 1 |
| ANXA5 | Annexin A5 | ACTR3C | Actin Related Protein 3C | ACTA1 | Actin Alpha 1, Skeletal Muscle | ENO2 | Enolase 2 |
| APCS | Amyloid P Component | AKNA | AT-Hook Transcription Factor | ACTA2 | Actin Alpha 2, Smooth Muscle | ENO3 | Enolase 3 |
| APOH | Apolipoprotein H | ALDH1L1 | Aldehyde Dehydrogenase 1 Family Member L1 | ACTB | Actin Beta | MYH16 | Myosin Heavy Chain 16 Pseudogene |
| ARHGAP21 | Rho GTPase Activating Protein 21 | C4A | Complement C4A (Rodgers Blood Group) | ACTBL2 | Actin Beta Like 2 | RPL7L1 | Ribosomal Protein L7 Like 1 |
| CFH | Complement Factor H | C4B_2 | Complement Component 4B | ACTC1 | Actin Alpha Cardiac Muscle 1 | SHLD3 | Shieldin Complex Subunit 3 |
| CHD8 | Chromodomain Helicase DNA Binding Protein 8 | C7orf57 | Complement C7 | ACTG1 | Actin Gamma 1 |
| CILP2 | Cartilage Intermediate Layer Protein | CAGE1 | Cancer Antigen 1 | ACTG2 | Actin Gamma 2, Smooth Muscle |
| CNOT6L | CCR4-NOT Transcription Complex Subunit 6 Like | CPSF2 | Cleavage And Polyadenylation Specific Factor 2 | ALB | Albumin |
| DAGLA | Diacylglycerol Lipase Alpha | DCAF4L2 | DDB1 And CUL4 Associated Factor 4 Like 2 | ANXA1 | Annexin A1 |
| Code     | Name                                      | Code     | Name                                      | Code | Name                                      | Code | Name                                      | X | X | Code     | Name                                      |
|----------|-------------------------------------------|----------|-------------------------------------------|------|-------------------------------------------|------|-------------------------------------------|---|---|----------|-------------------------------------------|
| DPYSL2   | Dihydropyrimidinase Like 2                | DHRS11   | Dehydrogenase/Reductase 11                |      | ANXA2                                      |      | Annexin A2                                |   |   |          |                                           |
| DPYSL3   | Dihydropyrimidinase Like 3                | DMD      | Dystrophin                                |      | ANXA2P2                                    |      | Annexin A2 Pseudogene 2                   |   |   |          |                                           |
| DYM      | Dymeclin                                  | FLNA     | Filamin A                                 |      | APOA1                                      |      | Apolipoprotein A1                          |   |   |          |                                           |
| DYN1H1   | Dynein Cytoplasmic 1 Heavy Chain          | HPR      | Haptoglobin-Related Protein               |      | ASPN                                        |      | Asporin                                    |   |   |          |                                           |
| ENPP3    | Ectonucleotide Pyrophosphatase/Phosphodiesterase 3 | HPX      | Hemopexin                                 |      | ATP5F1B                                     |      | ATP Synthase F1 Subunit Beta               |   |   |          |                                           |
| FGFR2    | Fibroblast Growth Factor Receptor 2       | IFT122   | Intraflagellar Transport 122              |      | BGN                                         |      | Biglycan                                   |   |   |          |                                           |
| GPSM2    | G Protein Signaling Modulator 2           | LMO7     | LIM Domain 7                              |      | C3                                          |      | Complement C3                              |   |   |          |                                           |
| GPX3     | Glutathione Peroxidase 3                  | MYO6     | Myosin VI                                 |      | CILP                                        |      | Cartilage Intermediate Layer Protein      |   |   |          |                                           |
| GSTP1    | Glutathione S-Transferase Pi 1            | PDIA3    | Protein Disulfide Isomerase Family A Member 3 |      | CLU                                         |      | Clusterin                                  |   |   |          |                                           |
| H2B1C    | H2B Clustered Histone 1                   | PPFIA1   | PTPRF Interacting Protein Alpha 1         |      | COL12A1                                     |      | Collagen Type XII Alpha 1 Chain            |   |   |          |                                           |
| H2BE1    | H2BE Variant Histone 1                    | PPFIA2   | PTPRF Interacting Protein Alpha 2         |      | COL14A1                                     |      | Collagen Type XIV Alpha 1 Chain            |   |   |          |                                           |
| HSPA1A   | Heat Shock Protein Family A (Hsp70) Member 1Å | PRDX1   | Peroxiredoxin 1                          |      | COL1A1                                      |      | Collagen Type I Alpha 1 Chain             |   |   |          |                                           |
## Table 2. Cont.

### Protein Expressed in Each Group of TMJ Synovial Fluid Sample (n = 225)

| Code | Name                          | Code | Name                          | Code | Name                          | X   | X   | Code | Name                          |
|------|-------------------------------|------|-------------------------------|------|-------------------------------|-----|-----|------|-------------------------------|
|      |                               |      |                               | DDWoR (n = 70) | MD (n = 34) | CH (n = 1) | DDWoR and MD (n = 114) | DDWoR and CH (n = 0) | MD and CH (n = 0) | DDWoR, MD and CH (n = 6) |
| HSPA1B | Heat Shock Protein Family A (Hsp70) Member 1B | PRDX2 | Peroxiredoxin 2 | COL6A1 | Collagen Type VI Alpha 1 Chain |
| HSPA1L | Heat Shock Protein Family A (Hsp70) Member 1 Like | RGMB | Repulsive Guidance Molecule BMP Co-Receptor B | COL6A2 | Collagen Type VI Alpha 2 Chain |
| HSPA2 | Heat Shock Protein Family A (Hsp70) Member 2 | SACM1L | SAC1 Like Phosphatidylinositide Phosphatase | COL6A3 | Collagen Type VI Alpha 3 Chain |
| HSPA8 | Heat Shock Protein Family A (Hsp70) Member 8 | SERPINA9 | Serpin Family A Member 9 | DCN | Decorin |
| IGLC1 | Immunoglobulin Lambda Constant 1 | SERPINH1 | Serpin Family H Member 1 | DES | Desmin |
| IGLC2 | Immunoglobulin Lambda Constant 2 | SLC4A1 | Solute Carrier Family 4 Member 1 | SMPD3 | Sphingomyelin Phosphodiesterase 3 |
| IGLC3 | Immunoglobulin Lambda Constant 3 | SMPD3 | Sphingomyelin Phosphodiesterase 3 | DPT | Dermatopontin |
| IGLC6 | Immunoglobulin Lambda Constant 6 | TENM4 | Transmembrane Protein 4 | FBN1 | Fibrinogen Alpha Chain |
| IGLC7 | Immunoglobulin Lambda Like Polypeptide 1 | TMTC3 | Testis Specific 10 | FGA | Fibrinogen Beta Chain |
| IGLL1 | Immunoglobulin Lambda Like Polypeptide 5 | TSGA10 | Transhyretin | FGB | Fibrinogen Gamma Chain |
| IGLL5 | Interferon Regulatory Factor 7 | TTR | Ubiquitin Specific Peptidase 10 | FGG | Fibromodulin |
| Code       | Name                                                | Code     | Name                          | Code   | Name                          | X | X | Code     | Name                |
|------------|-----------------------------------------------------|----------|-------------------------------|--------|-------------------------------|---|---|----------|---------------------|
| IRF7       | Kalirin RhoGEF Kinase                               | USP10    | Actin Related Protein 3B      | FMOD   | Fibronectin 1                 |   |   |          |                     |
| KALRN      | Kelch Repeat And BTB Domain Containing 11           |          |                               | FN1    | Glyceraldehyde-3-Phosphate Dehydrogenase |   |   |          |                     |
| KBTBD11    | Keratocan                                           | GAPDH    | Gelsolin                      |        |                                |   |   |          |                     |
| KERA       | Keratin 18                                          | GSN      | H2B Clustered Histone 11      |        |                                |   |   |          |                     |
| KRT18      | Keratin 7                                           | H2BC11   | H2B Clustered Histone 12      |        |                                |   |   |          |                     |
| KRT7       | Keratin 8                                           | H2BC12   | H2B Clustered Histone 13      |        |                                |   |   |          |                     |
| KRT8       | Keratin 84                                          | H2BC13   | H2B Clustered Histone 14      |        |                                |   |   |          |                     |
| KRT84      | Putative Uncharacterized Protein                     | H2BC14   | H2B Clustered Histone 15      |        |                                |   |   |          |                     |
| LOC400499  | Leucine Rich Repeat Containing 9                    | H2BC15   | H2B Clustered Histone 17      |        |                                |   |   |          |                     |
| LRRC9      | Mitogen-Activated Protein Kinase Kinase 7           | H2BC17   | H2B Clustered Histone 18      |        |                                |   |   |          |                     |
| MAP3K7     | Microfibril Associated Protein 5                    | H2BC18   | H2B Clustered Histone 21      |        |                                |   |   |          |                     |
| MFAP5      | Myosin Light Chain 6B                               | H2BC21   | H2B Clustered Histone 3       |        |                                |   |   |          |                     |
| MYL6B      | NCK Associated Protein 5                            | H2BC3    | H2B Clustered Histone 5       |        |                                |   |   |          |                     |
| NCKAP5     | Nik Related Kinase                                  | H2BC5    | H2B Clustered Histone 9       |        |                                |   |   |          |                     |
Table 2. Cont.

Protein Expressed in Each Group of TMJ Synovial Fluid Sample ($n = 225$)

| Protein Name                      | Code | Name                        | Code | Name                        | Code | Name                        | Code | Name                        | Code | Name                        | Code | Name                        | Code | Name                        |
|-----------------------------------|------|-----------------------------|------|-----------------------------|------|-----------------------------|------|-----------------------------|------|-----------------------------|------|-----------------------------|------|-----------------------------|
| NRK Pericentriolar Material 1    | NRK  | Pericentriolar Material 1   |      | H2BC9                       | H2BC9| H2B.S Histone 1             |      | X                           |      | X                           |      | X                           |      | X                           |
| PCM1 Procollagen C-Endopeptidase Enhancer | PCM1 | Procollagen C-Endopeptidase Enhancer | | H2BS1 | H2BS1| H2B.U Histone 1             | | X                           | | X                           | | X                           | | X                           |
| PCOLCE RAD54 Like                | PCOLCE | RAD54 Like                  | | H2BU1 | H2BU1| Hemoglobin Subunit Alpha 1  | | X                           | | X                           | | X                           | | X                           |
| RAD54L Retinol Dehydrogenase 5   | RAD54L | Retinol Dehydrogenase 5    | | HBA1 | HBA1| Hemoglobin Subunit Alpha 2  | | X                           | | X                           | | X                           | | X                           |
| RDH5 Ret Proto-Oncogene          | RDH5  | Ret Proto-Oncogene          | | HBA2 | HBA2| Hemoglobin Subunit Beta     | | X                           | | X                           | | X                           | | X                           |
| RET Regulatory Factor X1         | RET   | Regulatory Factor X1        | | HBB  | HBB | Hemoglobin Subunit Delta    | | X                           | | X                           | | X                           | | X                           |
| RFX1 RPTOR Independent Companion Of MTOR Complex 2 | RFX1 | RPTOR Independent Companion Of MTOR Complex 2 | | HBD  | HBD | Hemoglobin Subunit Epsilon 1 | | X                           | | X                           | | X                           | | X                           |
| RICTOR RIMS Binding Protein 3    | RICTOR | RIMS Binding Protein 3      | | HBE1 | HBE1| Hemoglobin Subunit Gamma 1  | | X                           | | X                           | | X                           | | X                           |
| RIMBP3 RUN And FYVE Domain Containing 2 | RIMBP3 | RUN And FYVE Domain Containing 2 | | HBG1 | HBG1| Hemoglobin Subunit Gamma 2  | | X                           | | X                           | | X                           | | X                           |
| RUFY2 Serpin Family C Member 1   | RUFY2 | Serpin Family C Member 1    | | HBG2 | HBG2| Haptoglobin                 | | X                           | | X                           | | X                           | | X                           |
| SERPINC1 Serpin Family F Member 1 | SERPINC1 | Serpin Family F Member 1   | | HP   | HP | Heat Shock Protein Family B (Small) Member 1 | | X                           | | X                           | | X                           | | X                           |
### Table 2. Cont.
Protein Expressed in Each Group of TMJ Synovial Fluid Sample (n = 225)

| Code     | Name                                      | Code     | Name                                      | Code     | Name                                      | Code     | Name                                      |
|----------|-------------------------------------------|----------|-------------------------------------------|----------|-------------------------------------------|----------|-------------------------------------------|
| DDWoR (n = 70) |                                       | MD (n = 34) |                                       | CH (n = 1) |                                       | DDWoR and MD (n = 114) |                                       | DDWoR and CH (n = 0) | MD and CH (n = 0) | DDWoR, MD and CH (n = 6) |
| SERPINF1 | SEC14 And Spectrin Domain Containing 1    | HSPB1    | Immunoglobulin Heavy Constant Alpha 1    | X         | X                                         |                      | X                                         |                      |                      |                      |
| SESTD1   | Small Nuclear Ribonucleoprotein U5 Subunit 200 | IGHA1    | Immunoglobulin Heavy Constant Alpha 2 (A2m Marker) | X         | X                                         |                      | X                                         |                      |                      |                      |
| SNRNP200 | SVOP Like                                 | IGHA2    | Immunoglobulin Heavy Constant Gamma 1 (G1m Marker) | X         | X                                         |                      | X                                         |                      |                      |                      |
| SVOPL    | Transcription Elongation Factor, Mitochondrial | IGHG1    | Immunoglobulin Heavy Constant Gamma 2    | X         | X                                         |                      | X                                         |                      |                      |                      |
| TEFM     | Thrombospondin 3                         | IGHG2    | Immunoglobulin Heavy Constant Gamma 3    | X         | X                                         |                      | X                                         |                      |                      |                      |
| THBS3    | Tenascin C                               | IGHG3    | Immunoglobulin Heavy Constant Gamma 4    | X         | X                                         |                      | X                                         |                      |                      |                      |
| TNC      | Trio Rho Guanine Nucleotide Exchange Factor | IGHG4    | Immunoglobulin Kappa Constant            | X         | X                                         |                      | X                                         |                      |                      |                      |
| TRIO     | Tubulin Beta 1 Class VI                  | IGKC     | Internexin Neuronal Intermediate Filament Protein Alpha | X         | X                                         |                      | X                                         |                      |                      |                      |
| DDWoR (n = 70) | MD (n = 34) | CH (n = 1) | DDWoR and MD (n = 114) | DDWoR and CH (n = 0) | MD and CH (n = 0) | DDWoR, MD and CH (n = 6) |
|---------------|-------------|------------|------------------------|----------------------|------------------|-----------------------|
| TUBB1         |             |            |                        |                      |                  |                       |
| Ubiquitin Specific Peptidase 42 |             |            |                        |                      |                  |                       |
| USP42         |             |            |                        |                      |                  |                       |
| WW Domain Binding Protein 1 Like |             |            |                        |                      |                  |                       |
| WBP1L         |             |            |                        |                      |                  |                       |
| Zinc Finger ZZ-Type And EF-Hand Domain Containing 1 |             |            |                        |                      |                  |                       |
| ZZEF1         |             |            |                        |                      |                  |                       |
| H2B Clustered Histone 1 |             |            |                        |                      |                  |                       |
|             |             |            |                        |                      |                  |                       |
| TUBB1         |             |            |                        |                      |                  |                       |
| Ubiquitin Specific Peptidase 42 |             |            |                        |                      |                  |                       |
| LGALS1        |             |            |                        |                      |                  |                       |
| WW Domain Binding Protein 1 Like |             |            |                        |                      |                  |                       |
| LMNA          |             |            |                        |                      |                  |                       |
| ZZEF1         |             |            |                        |                      |                  |                       |
| H2B Clustered Histone 1 |             |            |                        |                      |                  |                       |
| LUM           |             |            |                        | Microfibril Associated Protein 4 |                  |                       |
| MFAP4         |             |            |                        |                      |                  |                       |
| Myosin Light Chain 6 |             |            |                        |                      |                  |                       |
| MYL6          |             |            |                        |                      |                  |                       |
| Myocilin      |             |            |                        |                      |                  |                       |
| MYOC          |             |            |                        | Neurofilament Heavy |                  |                       |
| NEFH          |             |            |                        |                      |                  |                       |
| Neurofilament Light |             |            |                        |                      |                  |                       |
| NEFL          |             |            |                        |                      |                  |                       |
| Neurofilament Medium |             |            |                        |                      |                  |                       |
| NEFM          |             |            |                        |                      |                  |                       |
| Osteoglycin   |             |            |                        |                      |                  |                       |
| OGN           |             |            |                        | Pellino E3 Ubiquitin Protein Ligase Family Member 3 |                  |                       |
| PEI3          |             |            |                        |                      |                  |                       |
| Pyruvate Kinase M1/2 |             |            |                        |                      |                  |                       |
| PKM           |             |            |                        |                      |                  |                       |
| POTE Ankyrin Domain Family Member E |             |            |                        |                      |                  |                       |
Table 2. Cont.

| Protein Expressed in Each Group of TMJ Synovial Fluid Sample (n = 225) |
|---------------------------------------------------------------|
| **DDWoR (n = 70)** | **MD (n = 34)** | **CH (n = 1)** | **DDWoR and MD (n = 114)** | **DDWoR and CH (n = 0)** | **MD and CH (n = 0)** | **DDWoR, MD and CH (n = 6)** |
| POTEE | POT Ankyrin Domain Family Member F |  |  |  |  |  |
| POTEF | POT Ankyrin Domain Family Member I |  |  |  |  |  |
| POTEI | POT Ankyrin Domain Family Member J |  |  |  |  |  |
| POTEJ | POT Ankyrin Domain Family Member K, Pseudogene |  |  |  |  |  |
| POTEKp | Peptidylprolyl Isomerase A |  |  |  |  |  |
| PPIA | Proline And Arginine Rich End Leucine Rich Repeat Protein |  |  |  |  |  |
| PRELP | Peripherin |  |  |  |  |  |
| PRPH | S100 Calcium Binding Protein A10 |  |  |  |  |  |
| S100A10 | Serpin Family A Member 1 |  |  |  |  |  |
| SERPINA1 | Superoxide Dismutase 3 |  |  |  |  |  |
| SOD3 | Transferrin |  |  |  |  |  |
Table 2. Cont.

Protein Expressed in Each Group of TMJ Synovial Fluid Sample (n = 225)

| DDWoR (n = 70) | MD (n = 34) | CH (n = 1) | DDWoR and MD (n = 114) | DDWoR and CH (n = 0) | MD and CH (n = 0) | DDWoR, MD and CH (n = 6) |
|----------------|-------------|------------|------------------------|----------------------|------------------|----------------------|
| TF | Transforming Growth Factor Beta Induced | Thrombospondin 4 |
| TGFB1 | THBS4 | Tenascin XA |
| TNXA | Tenascin XB |
| TNXB | Tubulin Alpha 1a |
| TUBA1A | Tubulin Alpha 1b |
| TUBA1B | Tubulin Alpha 1c |
| TUBA1C | Tubulin Alpha 3c |
| TUBA3C | Tubulin Alpha 3d |
| TUBA3D | Tubulin Alpha 3e |
| TUBA3E | Tubulin Alpha 4a |
| TUBA4A | Tubulin Alpha 8 |
| TUBA6 | Tubulin Beta Class I |
| TUBB | Tubulin Beta 2A Class IIa |
| TUBB2A | Tubulin Beta 2B Class IIb |
| TUBB2B | Tubulin Beta 3 Class III |
| TUBB3 | Tubulin Beta 4A Class IVa |
### Table 2. Cont.

**Protein Expressed in Each Group of TMJ Synovial Fluid Sample (n = 225)**

| Group                                      | Protein Name | Code      |
|--------------------------------------------|--------------|-----------|
| DDWoR (n = 70)                             | TUBB4A       | TUBB4A    |
| MD (n = 34)                                | TUBB4B       | TUBB4B    |
| CH (n = 1)                                 | TUBB6        | TUBB6     |
| DDWoR and MD (n = 114)                     | TUBB8        | TUBB8     |
| DDWoR and CH (n = 0)                       | TUBB8B       | TUBB8B    |
| DDWoR and MD (n = 114)                     | VCAN         | VCAN      |
| DDWoR and CH (n = 0)                       | VIM          | VIM       |
| DDWoR, MD and CH (n = 6)                   |              |           |

### Table 3.

**Gene code and name of the proteins expressed in temporomandibular joint (TMJ) discs of all groups (DDWoR, MD, CH) and between the groups DDWoR and MD, DDWoR and CH, MD and CH and DDWoR, MD and CH.**

| Code | Name                      | Code | Name                      | Code | Name                      | Code | Name                      | Code | Name                      | Code | Name                      | Code | Name                      |
|------|---------------------------|------|---------------------------|------|---------------------------|------|---------------------------|------|---------------------------|------|---------------------------|------|---------------------------|
| ABCC9| ATP Binding Cassette      | AFTH | Aftiphilin                | ACTN1| Actinin Alpha 1           | ATP7B| ATPase Copper Transporting Beta |
| ACSS3| Acyl-CoA Synthetase       | AKAP13| A-kinase anchor protein 13| ACTN4| Actinin Alpha 4           | AXIN2| Axin 2                    |
| AGO4 | Argonaute RISC Component 4| ALDHJ1A2| Aldehyde dehydrogenase family 3 member A2| ACTR3| Actin Related Protein 3   | C4A   | Complement C4A            | BRD3 | Bromodomain Containing 3 | KRT3 | Keratin 3                | ACTA1| Actin Alpha 1, Skeletal Muscle |
| Code     | Name                          | Code     | Name                          | Code     | Name                          | Code     | Name                          | Code     | Name                          | Code     | Name                          | Code     | Name                          |
|----------|-------------------------------|----------|-------------------------------|----------|-------------------------------|----------|-------------------------------|----------|-------------------------------|----------|-------------------------------|----------|-------------------------------|
| AMBP     | Alpha-1-Microglobulin/Bikunin Precursor | ANKRD44  | Serine/threonine-protein phosphatase 6 regulatory ankyrin repeat subunit B | ADAM10   | ADAM Metalloprotease Domain 10 | C4B      | Complement C4B                | CLTC     | Clathrin Heavy Chain          | KRT5     | Keratin 5                     | ACTA2    | Actin Alpha 2, Smooth Muscle  |
| ANKRD17  | Ankyrin Repeat Domain 17      | ANKRD52  | Serine/threonine-protein phosphatase 6 regulatory ankyrin repeat subunit C | ADSL     | Adenylosuccinate Lyase        | C4B_2    | Complement Component 4B       | COL1A1   | Collagen Type 1 Alpha 1 Chain | KRT6A    | Keratin 6A                    | ACTB     | Actin Beta                    |
| ARHGAP35 | Rho GTPase Activating Protein 35 | ARMH3    | Armadillo-like helical domain-containing protein 3 | ALDOA    | Aldolase, Fructose-Bisphosphate A | KERA     | Keratocan                     | COL4A6   | Collagen Type IV Alpha 6 Chain | KRT6B    | Keratin 6B                    | ACTBL2   | Actin Beta Like 2             |
| ARHGEF10 | Rho Guanine Nucleotide Exchange Factor 10 | CCDC88A  | Girdin                        | ALDOC    | Aldolase, Fructose-Bisphosphate C | KIAA0556 | Katanin Interacting Protein   | DNAH8    | Defensin Alpha 1              | KRT6C    | Keratin 6C                    | ACTC1    | Actin Alpha Cardiac Muscle 1  |
| ATAD2B   | ATPase Family AAA Domain Containing 2B | CLUH     | Clustered mitochondria protein homolog | ANKMY1   | Ankyrin Repeat And MYND Domain Containing 1 | MAP4     | Microtubule Associated Protein 4 | EEF1A1   | Dynein Axonemal Heavy Chain 8 | KRT75    | Keratin 75                    | ACTG1    | Actin Gamma 1                 |
| BCAS2    | BCAS2 Pre-MRNA Processing Factor | COL4A1   | Collagen alpha-II(V) chain    | ANXA5    | Annexin A5                    | SEMA4F   | Semaphorin 4F                 | EEF1A1P5 | Eukaryotic Translation Elongation Factor 1 Alpha 1 | KRT76    | Keratin 76                    | ACTG2    | Actin Gamma 2                 |
| CARN51   | Carnosine Synthase 1          | DOCK10   | Deducator of cytokinesis protein 10 | ANXA6    | Annexin A6                    | EEF1A2   | Eukaryotic Translation Elongation Factor 1 Alpha 1 | KRT78    | Keratin 78                    | ALB      | Albumin                       |
| CCDC187  | Coiled-Coil Domain Containing 187 | DTHD1    | Death domain-containing protein 1 | ASXL1    | ASXL Transcriptional Regulator 1 | HMCN2    | Eukaryotic Translation Elongation Factor 1 Alpha 2 | KRT79    | Keratin 79                    | ANXA1    | Annexin A1                    |
Table 3. Cont.

| Code | Name                          | Code | Name                          | Code | Name                          | Code | Name                          | Code | Name                          | Code | Name                          |
|------|-------------------------------|------|-------------------------------|------|-------------------------------|------|-------------------------------|------|-------------------------------|------|-------------------------------|
|      |                                |      |                                |      |                                |      |                                |      |                                |      |                                |
| DDoWR (n = 66) |                                | MD (n = 38) |                                | CH (n = 89) |                                | DDoWR and MD (n = 9) |                                | DDoWR and CH (n = 28) |                                | MD and CH (n = 17) | DDoWR, MD and CH (n = 132) |
| CDCP1 | CUB Domain Containing Protein 1   | ERAS | GTPase ERas                   | ATP2C1 | ATPase Secretory Pathway Ca2+ Transporting 1 | HSPA2 | Hemicentin 2               | KRT81 | Keratin 81                 | ANXA2 | Annexin A2                    |
| CDH3  | Cadherin 3                     | ERBIN | Erbin                         | BLOC1S1 | Biogenesis Of Lyosomal Organelles Complex 1 Subunit 1 | HSPA8 | Heat Shock Protein Family A (Hsp70) Member 2 | KRT83 | Keratin 83                 | ANXA2P2 | Annexin A2 Pseudogene 2     |
| CHD7  | Chromodomain Helicase DNA Binding Protein 7 | FLNA | Filamin-A                     | BRCA2 | BRCA2 DNA Repair Associated | HYDIN | Heat Shock Protein Family A (Hsp70) Member 8 | KRT85 | Keratin 85                 | APOA1 | Apolipoprotein A1            |
| CHD8  | Chromodomain Helicase DNA Binding Protein 8 | GOT1L1 | Putative aspartate aminotransferase, cytoplasmic 2 | CMBP5 | Calcium Binding Protein 5     | IGLC1 | HYDIN Axonemal Central Pair Apparatus Protein | KRT86 | Keratin 86                 |        |                               |
| CHD9  | Chromodomain Helicase DNA Binding Protein 9 | HHHL1 | HERV-H LTR-associating protein 1 | CACNA2D3 | Calcium Voltage-Gated Channel Auxiliary Subunit Alpha2delta 3 | IGLC2 | Immunoglobulin Lambda Constant 1 | PKM   | Pyruvate Kinase M1/2       | APOC1 | Apolipoprotein C1            |
| CSTF2T | Cleavage Stimulation Factor Subunit 2 Tau Variant | IGHV3OR16–9 | Immunoglobulin heavy variable 3/OR16–9 (non-functional) | CDC18 | Coiled-Coil Domain Containing 18 | IGLC3 | Immunoglobulin Lambda Constant 2 | TTBK2 | Tau Tubulin Kinase 2       | BGN   | Biglycan                      |
| ECH1  | Enoyl-CoA Hydratase 1          | KDF1 | Keratinocyte differentiation factor 1 | CDC20 | Cell Division Cycle 20        | IGLC6 | Immunoglobulin Lambda Constant 3 | C3    | Complement C3               | CILP  | Cartilage Intermediate Layer Protein |
| ELAVL3 | ELAV Like RNA Binding Protein 3 | L1CAM | Neural cell adhesion molecule L1 | CENPF | Centromere Protein F          | IGLC7 | Immunoglobulin Lambda Constant 6 | CILP  | Cartilage Intermediate Layer Protein |
| EML4  | EMA Like 4                     | MARK1 | Serine/threonine-protein kinase MARK1 | CFAP20DC | Domain Containing              | IGLL1 | Immunoglobulin Lambda Constant 7 | CILP2 | Cartilage Intermediate Layer Protein 2 |        |                               |
| FARP2 | FERM, ARH/RhoGEF And Pleckstrin Domain Protein 2 | NEIL3 | Endonuclease 8-like 3         | CNTN1 | Contactin 1                   | IGLL5 | Immunoglobulin Lambda Like Polypeptide 1 | CLU   | Clusterin                    |        |                               |
# Table 3. Cont.

| Code  | Name                                | Code   | Name                                | Code    | Name                        | Code    | Name                                | Code    | Name                                | Code    | Name                                | Code    | Name                                |
|-------|-------------------------------------|--------|-------------------------------------|---------|-----------------------------|---------|-------------------------------------|---------|-------------------------------------|---------|-------------------------------------|---------|-------------------------------------|
| FBN1  | Fibrillin 1                         | NOL8   | Nucleolar protein 8                 | COQ8B   | Coenzyme Q8B                | LOC441081 | Immunoglobulin Lambda Like Polypeptide 5 | COL12A1 | Collagen Type XII Alpha 1 Chain    |
| GALK2 | Galactokinase 2                     | NUFIP1 | Nuclear fragile X mental retardation-interacting protein 1 | CTNNA3 | Catennin Alpha 3            | MIS18BP1 | POM121 Membrane Glycoprotein (Rat) Pseudogene | COL14A1 | Collagen Type XIV Alpha 1 Chain    |
| GPR162| G Protein-Coupled Receptor 162      | NUMA1  | Nuclear mitotic apparatus protein 1 | DPYSL2  | Dihydropyrimidinase Like 2 | MYO15B | MB18 Binding Protein 1               | COL6A1 | Collagen Type VI Alpha 1 Chain    |
| GPRAS1| G Protein-Coupled Receptor Associated Sorting Protein 1 | PARP10 | Protein mono-ADP-ribosyltransferase PARP10 | EHD2 | EH Domain Containing 2 | POSTN | Myosin XV B                         | COL6A2 | Collagen Type VI Alpha 2 Chain    |
| IKBKE | Inhibitor Of Nuclear Factor Kappa B Kinase Subunit Epsilon | PCDHA4 | Protocadherin alpha-4               | EYS     | Eyes Shut Homolog           | SERPINA9 | Peristin                            | COL6A3 | Collagen Type VI Alpha 3 Chain    |
| INS   | Insulin                             | POLD1  | DNA polymerase delta catalytic subunit | FI3A1 | Coagulation Factor XII A Chain | VTN | Serpin Family A Member 9            | COMP   | Cartilage Oligomeric Matrix Protein |
| IRF2BP1| Interferon Regulatory Factor 2 Binding Protein Like | POM121L2 | POM121-like protein 2 | GOLGA4 | Golgin A4                   | G13A1   | Glutathione S-Transferase Pi 1       | DCN    | Decorin                            |
| ITGA6 | Integrin Subunit Alpha 6            | PFIA1  | Liprin-alpha-1                      | GSTP1   | Glutathione S-Transferase Pi 1 | P1      | DMD                                | DES    | Desmin                             |
| KRT26 | Keratin 26                          | PFIA2  | Liprin-alpha-2                      | GV1NP1  | GTPase, Very Large Interferon Inducible Pseudogene 1 | DMD    | Dystrophin                          |
| LEMD2 | LEM Domain Nuclear Envelope Protein 2 | PRR14L | Protein PRR14L                      | H3-2    | H3.2 Histone (Putative)     | H3-3A   | H3.3 Histone A                      | DPT    | Dermatopontin                      |
| MAP3K21| Mitogen-Activated Protein Kinase Kinase Kinase 21 | PTPN7 | Tyrosine-protein phosphatase non-receptor type 7 | H3-3A   | H3.3 Histone A              | ENO1    | Enolase 1                          |

**Protein Expressed in Each Group of TMJ Disc Sample (n = 379)**

- DDWoR (n = 66)
- MD (n = 38)
- CH (n = 89)
- DDWoR and MD (n = 9)
- DDWoR and CH (n = 28)
- MD and CH (n = 17)
- DDWoR, MD and CH (n = 132)
Table 3. Cont.

Protein Expressed in Each Group of TMJ Disc Sample (n = 379)

| Code | Name                                      | Code | Name                                      | Code | Name                                      | Code | Name                                      | Code | Name                                      | Code | Name                                      |
|------|-------------------------------------------|------|-------------------------------------------|------|-------------------------------------------|------|-------------------------------------------|------|-------------------------------------------|------|-------------------------------------------|
| MDGA1| MAM Domain Containing Glycosylphosph-      |      | RASSF10                                   |      | H3-3B H3.3 Histone B                     |      | ENO2 Enolase 2                            |      |                                           |      |                                           |
|      | phatidylinositol Anchor 1                 |      |                                           |      |                                           |      |                                           |      |                                           |      |                                           |
| MMP10| Matrix Metallopeptidase 10                |      | RPS6KA6                                   |      | H3-4 H3.4 Histone                        |      | ENO3 Enolase 3                            |      |                                           |      |                                           |
| MMP27| Matrix Metallopeptidase 27                |      | TRIO                                      |      | H3-5 H3.5 Histone                        |      | FBLN1 Fibulin 1                           |      |                                           |      |                                           |
| MMP3 | Matrix Metallopeptidase 3                 |      | TSC1                                      |      | HEAT Repeat Containing 6                 |      | FGA Fibrinogen Alpha Chain                |      |                                           |      |                                           |
| MOS  | MOS Proto-Oncogene, Serine/Threonine      |      | UPK3A                                     |      | HPX Hemopexin                            |      | FGB Fibrinogen Beta Chain                 |      |                                           |      |                                           |
|      | Kinases                                   |      |                                           |      |                                           |      |                                           |      |                                           |      |                                           |
| MYL6 | Myosin Light Chain 6                     |      | UROD                                      |      | Heat Shock Protein Family 90 Beta Family Member 1 |      | FGG Fibrinogen Gamma Chain                |      |                                           |      |                                           |
| MYO7B| Myosin VIIB                               |      | HSPA1A                                    |      | Heat Shock Protein Family A (Hsp70) Member 1A |      | FLNB Filamin B                            |      |                                           |      |                                           |
| NT5E | 5'-Nucleotidase Ecto                     |      | HSPA1B                                    |      | Heat Shock Protein Family A (Hsp70) Member 1B |      | FMOD Fibromodulin                         |      |                                           |      |                                           |
| OLFML1| Olfactomedin Like 1                      |      | HSPA1L                                    |      | Heat Shock Protein Family A (Hsp70) Member 1 Like |      | FN1 Fibronectin 1                          |      |                                           |      |                                           |
| PGM5 | Phosphoglhummatase 5                     |      | HSPA5                                     |      | Heat Shock Protein Family A (Hsp70) Member 5 |      | GAPDH Glyceraldehyde-3-Phosphate Dehydrogenase |      |                                           |      |                                           |
### Table 3. Cont.

**Protein Expressed in Each Group of TMJ Disc Sample (n = 379)**

| Code     | Name                                      | Code     | Name                                      | Code     | Name                                      | Code     | Name                                      | Code     | Name                                      | Code     | Name                                      | Code     | Name                                      | Code     | Name                                      |
|----------|-------------------------------------------|----------|-------------------------------------------|----------|-------------------------------------------|----------|-------------------------------------------|----------|-------------------------------------------|----------|-------------------------------------------|----------|-------------------------------------------|----------|-------------------------------------------|
| PHKA2    | Phosphorylase Kinase Regulatory Subunit    | IGFN1    | Immunoglobulin Like And Fibronectin Type  | GPX3     | Glutathione Peroxidase 3                 |          |                                           |          |                                           |          |                                           |          |                                           |          |                                           |
|          | Alpha 2                                   |          | III Domain Containing 1                   |          |                                           |          |                                           |          |                                           |          |                                           |          |                                           |          |                                           |
| PLA2G7   | Phospholipase A2                          | INF2     | Inverted Formin 2                         | GSN      | Angiotensin I Converting Enzyme 2        |          |                                           |          |                                           |          |                                           |          |                                           |          |                                           |
|          | Group VII                                 |          |                                           |          |                                           |          |                                           |          |                                           |          |                                           |          |                                           |          |                                           |
| POR      | Cytochrome P450                           | L3MBTL4  | L3MBTL Histone Methyl-Lysine Binding      | H2BC1    | H2B Clustered Histone 1                  |          |                                           |          |                                           |          |                                           |          |                                           |          |                                           |
|          | Oxidoreductase                            |          | Protein 4                                | H2BC11   | H2B Clustered Histone 11                 |          |                                           |          |                                           |          |                                           |          |                                           |          |                                           |
| RANBP17  | RAN Binding Protein 17                    | LMNB1    | Lamin B1                                 | H2BC12   | H2B Clustered Histone 12                 |          |                                           |          |                                           |          |                                           |          |                                           |          |                                           |
| RCS22    | Regulator Of G Protein Signaling 22       | LMNB2    | Lamin B2                                 | H2BC13   | H2B Clustered Histone 13                 |          |                                           |          |                                           |          |                                           |          |                                           |          |                                           |
| RIF1     | Replication Timing Regulatory Factor 1    | MFA5     | Microfilibr Associated Protein 5          | H2BC14   | H2B Clustered Histone 14                 |          |                                           |          |                                           |          |                                           |          |                                           |          |                                           |
| RTN4     | Reticulon 4                               | MRPL50   | Mitochondrial Ribosomal Protein L50       | H2BC15   | H2B Clustered Histone 15                 |          |                                           |          |                                           |          |                                           |          |                                           |          |                                           |
| SARS2    | Seryl-TRNA Synthetase 2, Mitochondrial    | MS4A6A   | Membrane Spanning 4-Domains A6A           | H2BC17   | H2B Clustered Histone 17                 |          |                                           |          |                                           |          |                                           |          |                                           |          |                                           |
| SEPHS2   | Selenophosphate Synthetase 2              | MUC4     | Mucin 4, Cell Surface Associated          | H2BC18   | H2B Clustered Histone 18                 |          |                                           |          |                                           |          |                                           |          |                                           |          |                                           |
| SLFN13   | Schlaen Family Member 13                  | MYH14    | Myosin Heavy Chain 14                    | H2BC21   | H2B Clustered Histone 21                 |          |                                           |          |                                           |          |                                           |          |                                           |          |                                           |
| SLK      | STE20 Like Kinase                         | MYL6B    | Myosin Light Chain 6B                    | H2BC23   | H2B Clustered Histone 3                  |          |                                           |          |                                           |          |                                           |          |                                           |          |                                           |
| SPATA20  | Spermatogenesis Associated 20             | NEK10    | NIMA Related Kinase 10                   | H2BC3    | H2B Clustered Histone 3                  |          |                                           |          |                                           |          |                                           |          |                                           |          |                                           |
| SPATA5   | Spermatogenesis Associated 5              | PAK3     | P21 (RAC1) Activated Kinase 3            | H2BC5    | H2B Clustered Histone 5                  |          |                                           |          |                                           |          |                                           |          |                                           |          |                                           |
### Table 3. Cont.

Protein Expressed in Each Group of TMJ Disc Sample (*n* = 379)

| Code       | Name                                      | Code       | Name                                      | Code       | Name                                      | Code       | Name                                      | Code       | Name                                      |
|------------|-------------------------------------------|------------|-------------------------------------------|------------|-------------------------------------------|------------|-------------------------------------------|------------|-------------------------------------------|
| DDWoR      | MD (*n* = 38)                              | CH (*n* = 89) | DDWoR and MD (*n* = 9) | DDWoR and CH (*n* = 28) | MD and CH (*n* = 17) | DDWoR, MD and CH (*n* = 132) |
| **SPTA1**  | Spectrin Alpha, Erythrocytic 1            | **PAPOLA** | Poly(A) Polymerase Alpha                  | **H2BC9**  | H2B Clustered Histone 9                  |
| **SQUE**   | Squalene Epoxidase                        | **PAPOLG** | Poly(A) Polymerase Gamma                  | **H2BS1**  | H2B-S Histone 1                          |
| **ST20-AS1** | ST20 Antisense RNA 1                      | **PDIA3**  | Protein Disulfide Isomerase Family A Member 3 | **H2BU1**  | H2B-U Histone 1                          |
| **STIL**   | STIL Centriolar Assembly Protein          | **PDLIM4** | PDZ And LIM Domain 1                     | **HBA1**   | Hemoglobin Subunit Alpha 1               |
| **TACC2**  | Transforming Acidic Coiled-Coil Containing Protein 2 | **RALBP1** | RaLA Binding Protein 1                   | **HBA2**   | Hemoglobin Subunit Alpha 2               |
| **TAPI**   | Transporter 1, ATP Binding Cassette Subfamily B Member | **RNF213** | Ring Finger Protein 213                  | **HBB**    | Hemoglobin Subunit Beta                 |
| **THADA**  | THADA Armadillo Repeat Containing         | **SBF2**   | SET Binding Factor 2                     | **HBD**    | Hemoglobin Subunit Delta                 |
| **THBS3**  | Thrombospondin 3                          | **SERPINF1** | Serpin Family F Member 1                 | **HBE1**   | Hemoglobin Subunit Epsilon 1            |
| **UQCRC1** | Ubiquinol-Cytochrome C Reductase Core Protein 1 | **SERPINH1** | Serpin Family H Member 1                | **HBG1**   | Hemoglobin Subunit Gamma 1              |
| **VWA3A**  | Von Willebrand Factor A Domain Containing 3A | **SLC4A5** | Solute Carrier Family 4 Member 5         | **HBG2**   | Hemoglobin Subunit Gamma 2              |
| **ZNF333** | Zinc Finger Protein 333                   | **SLIT2**  | Slit Guidance Ligand 2                   | **HBZ**    | Hemoglobin Subunit Zeta                 |
| **SMPD3**  | Sphingomyelin Phosphodiesterase 3         | **HP**     | Haptoglobin                             |
| **TAPT1**  | Transmembrane Anterior Posterior Trans-formation 1 | **HPR**   | Haptoglobin-Related Protein             |
Table 3. Cont.

Protein Expressed in Each Group of TMJ Disc Sample (n = 379)

| Code | Name                  | Code | Name                  | Code | Name                  | Code | Name                  | Code | Name                  | Code | Name                  |
|------|-----------------------|------|-----------------------|------|-----------------------|------|-----------------------|------|-----------------------|------|-----------------------|
| TBX22| T-Box Transcription Factor 22 |      |                       |      |                       |      |                       |      |                       |      |                       |
| TDRD1| Tudor Domain Containing 1 |      |                       |      |                       |      |                       |      |                       |      |                       |
| TENM4| Teneurin Transmembrane Protein 4 |      |                       |      |                       |      |                       |      |                       |      |                       |
| THBS1| Thrombospondin 1       |      |                       |      |                       |      |                       |      |                       |      |                       |
| TJP2 | Tight Junction Protein 2 |      |                       |      |                       |      |                       |      |                       |      |                       |
| TTR | Transthyretin           |      |                       |      |                       |      |                       |      |                       |      |                       |
| UBP1 | Upstream Binding Protein 1 |      |                       |      |                       |      |                       |      |                       |      |                       |
| WHRN | Whirlin                |      |                       |      |                       |      |                       |      |                       |      |                       |
| ZNF155| Zinc Finger Protein 155 |      |                       |      |                       |      |                       |      |                       |      |                       |
| ZNF221| Zinc Finger Protein 221 |      |                       |      |                       |      |                       |      |                       |      |                       |

DDWoR (n=66) | MD (n=38) | CH (n=89) | DDWoR and MD (n=9) | DDWoR and CH (n=28) | MD and CH (n=17) | DDWoR, MD and CH (n=132)
Table 3. Cont.

Protein Expressed in Each Group of TMJ Disc Sample (n = 379)

| Code  | Name                  | Code  | Name                  | Code  | Name                  | Code  | Name                  | Code  | Name                  | Code  | Name                  |
|-------|-----------------------|-------|-----------------------|-------|-----------------------|-------|-----------------------|-------|-----------------------|-------|-----------------------|
|       |                       |       |                       |       |                       |       |                       |       |                       |       |                       |
|       | **DDWoR** (n = 66)    | **MD** (n = 38) | **CH** (n = 89) | **DDWoR and MD** (n = 9) | **DDWoR and CH** (n = 28) | **MD and CH** (n = 17) | **DDWoR, MD and CH** (n = 132) |
|       |                       |       |                       |       |                       |       |                       |       |                       |       |                       |
| LMNA  | Lamin A/C             |       |                       |       |                       |       |                       |       |                       |       |                       |
| LUM   | Lumican               |       |                       |       |                       |       |                       |       |                       |       |                       |
| MFAP4 | Microfibril Associated Protein 4 | | | | | | | | | |
| MFGF8 | Milk Fat Globule EGF And Factor V/VII Domain Containing | | | | | | | | |
| MYH16 | Myosin Heavy Chain 16 Pseudogene | | | | | | | | |
| MYOC  | Myocilin              |       |                       |       |                       |       |                       |       |                       |       |                       |
| NEFH  | Neurofilament Heavy   |       |                       |       |                       |       |                       |       |                       |       |                       |
| NEFL  | Neurofilament Light   |       |                       |       |                       |       |                       |       |                       |       |                       |
| NEFM  | Neurofilament Medium  |       |                       |       |                       |       |                       |       |                       |       |                       |
| OGN   | Osteoglycin           |       |                       |       |                       |       |                       |       |                       |       |                       |
| POTEE | POTE Ankyrin Domain Family Member E | | | | | | | | |
| POTEF | POTE Ankyrin Domain Family Member F | | | | | | | | |
| POTEI | POTE Ankyrin Domain Family Member I | | | | | | | | |
| POTEJ | POTE Ankyrin Domain Family Member J | | | | | | | | |
| POTEKP| POTE Ankyrin Domain Family Member K, Pseudogene | | | | | | | | |
| PPIA  | Peptidylprolyl Isomerase A | | | | | | | | |
### Table 3. Cont.

Protein Expressed in Each Group of TMJ Disc Sample (*n* = 379)

| DDWoR (n = 66) | MD (n = 38) | CH (n = 89) | DDWoR and MD (n = 9) | DDWoR and CH (n = 28) | MD and CH (n = 17) | DDWoR, MD and CH (n = 132) |
|----------------|-------------|-------------|----------------------|-----------------------|---------------------|--------------------------|
| Code | Name | Code | Name | Code | Name | Code | Name | Code | Name | Code | Name |
| PRDX1 | Peroxiredoxin 1 | PRDX2 | Peroxiredoxin 2 | PRELP | Proline And Arginine Rich End Leucine Rich Repeat Protein | PRPH | Peripherin | RPL7L1 | Ribosomal Protein L7 Like 1 | S100A10 | S100 Calcium Binding Protein A10 |
| SALL3 | Spalt Like Transcription Factor 3 | SERPINA1 | Serpin Family A Member | SHLD3 | Shieldin Complex Subunit 3 | SLC4A1 | Solute Carrier Family 4 Member 1 | SOD3 | Superoxide Dismutase 3 | TF | Transferrin |
| TGFB1 | Transforming Growth Factor Beta Induced | THBS4 | Thrombospondin 4 | TNC | Tenascin C | TNXA | Tenascin XA (Pseudogene) | TNXB | Tenascin XB | TUBA1A | Tubulin Alpha 1a |
Table 3. Cont.

Protein Expressed in Each Group of TMJ Disc Sample (n = 379)

| Code | Name                  | Code   | Name                  | Code   | Name                  | Code   | Name                  | Code   | Name                  | Code   | Name                  |
|------|-----------------------|--------|-----------------------|--------|-----------------------|--------|-----------------------|--------|-----------------------|--------|-----------------------|
|      | DDWoR (n = 66)        |        |                       |        |                       |        |                       |        |                       |        |                       |
|      | MD (n = 38)           |        |                       |        |                       |        |                       |        |                       |        |                       |
|      | CH (n = 89)           |        |                       |        |                       |        |                       |        |                       |        |                       |
|      | DDWoR and MD (n = 9)  |        |                       |        |                       |        |                       |        |                       |        |                       |
|      | DDWoR and CH (n = 28) |        |                       |        |                       |        |                       |        |                       |        |                       |
|      | MD and CH (n = 17)    |        |                       |        |                       |        |                       |        |                       |        |                       |
|      | DDWoR, MD and CH      |        |                       |        |                       |        |                       |        |                       |        |                       |
|      | (n = 132)             |        |                       |        |                       |        |                       |        |                       |        |                       |
| TUBA1B | Tubulin Alpha 1b          |        |                       |        |                       |        |                       |        |                       |        |                       |
| TUBA1C | Tubulin Alpha 1c          |        |                       |        |                       |        |                       |        |                       |        |                       |
| TUBA3E | Tubulin Alpha 3e          |        |                       |        |                       |        |                       |        |                       |        |                       |
| TUBA4A | Tubulin Alpha 4a          |        |                       |        |                       |        |                       |        |                       |        |                       |
| TUBA5  | Tubulin Alpha 8           |        |                       |        |                       |        |                       |        |                       |        |                       |
| TUBB   | Tubulin Beta            |        | Class I               |        |                       |        |                       |        |                       |        |                       |
| TUBB1  | Tubulin Beta 1           |        | Class VI              |        |                       |        |                       |        |                       |        |                       |
| TUBB2A | Tubulin Beta 2A          |        | Class IIa             |        |                       |        |                       |        |                       |        |                       |
| TUBB2B | Tubulin Beta 2B          |        | Class IIb             |        |                       |        |                       |        |                       |        |                       |
| TUBB3  | Tubulin Beta 3           |        | Class III             |        |                       |        |                       |        |                       |        |                       |
| TUBB4A | Tubulin Beta 4A          |        | Class IVa             |        |                       |        |                       |        |                       |        |                       |
| TUBB4B | Tubulin Beta 4B          |        | Class IVb             |        |                       |        |                       |        |                       |        |                       |
| TUBB6  | Tubulin Beta 6           |        | Class V               |        |                       |        |                       |        |                       |        |                       |
| TUBB8  | Tubulin Beta 8           |        | Class VIII            |        |                       |        |                       |        |                       |        |                       |
| TUBB8B | Tubulin Beta 8B          |        |                       |        |                       |        |                       |        |                       |        |                       |
Table 4. Proteins expressed in both synovial fluid and TMJ disc samples of each group.

| Protein Expressed in Each Group of TMJ Synovial Fluid and Disc Samples (n = 11) |
|---------------------------------|---------|---------|-----------------|---------|---------|---------|
| DDWoR (n= 2)                   | MD (n= 3) | CH (n= 0) | DDWoR and MD (n= 0) | DDWoR and CH (n= 0) | MD and CH (n= 0) | DDWoR, MD and CH (n= 0) |
| CHD8                           | FLNA     | PPFIA1   | PPFIA2           | ENO1    | ENO2    | ENO3    |
| MYL6B                          | MYH16    | RPL7L1   | SHLD3            |         |         |         |

Table 5. Gene code, protein name and function for each sample of TMJ synovial fluid.

| Synovial Fluid Sample | Code          | Name                        | Function                                                   |
|-----------------------|---------------|-----------------------------|------------------------------------------------------------|
| DDWoR                | A2M           | Alpha-2-Macroglobulin       | Inhibits inflammatory cytokines.                           |
|                      | APCS          | Amyloid P Component, Serum  | Binds to apoptotic cells at an early stage.                |
|                      | GPSM2         | G Protein Signaling Modulator 2 | Involved in the development of normal hearing.         |
|                      | KRT18         | Keratin 18                  | Is involved in interleukin-6-mediated barrier protection. |
|                      | MAP3K7        | Mitogen-Activated Protein Kinase Kinase 7 | Mediates signal transduction various cytokines including interleukin-1, transforming growth factor-beta, bone morphogenetic protein 2 and 4, Toll-like receptors, tumor necrosis factor receptor CD40 and B-cell receptor. |
|                      | SERPINC1      | Serpin Family C Member 1    | This protein inhibits thrombin and it regulates the blood coagulation cascade. |
|                      | ALDH1L1       | Aldehyde Dehydrogenase 1 Family Member L1 | Associated with decreased apoptosis, increased cell motility, and cancer progression. |
|                      | C4A           | Complement C4A (Rodgers Blood Group) | An antimicrobial peptide and a mediator of local inflammation. |
|                      | HPX           | Hemopexin                   | Acute phase protein that transports heme from the plasma to the liver and may be involved in protecting cells from oxidative stress. |
|                      | IFT122        | Intraflagellar Transport 122 | Involved in cell cycle progression, signal transduction, apoptosis, and gene regulation. |
|                      | MYO6          | Myosin VI                   | This protein maintains the structural integrity of inner ear hair cells and mutations in this gene cause hearing loss. |
|                      | PRDX1         | Peroxiredoxin 1             | Has an antioxidant protective role in cells and may contribute to the antiviral activity of CD8(+) T-cells. |
|                      | SERPINH1      | Serpin Family H Member 1    | Plays a role in collagen biosynthesis as a collagen-specific molecular chaperone. |
|                      | SMPD3         | Sphingomyelin Phosphodiesterase 3 | Mediates cellular functions, such as apoptosis and growth arrest. |
|                      | ADH1          | Alcohol Dehydrogenase Subunit Alpha | Catalyzes the oxidation of alcohols to aldehydes. |
|                      | ANXA1         | Annexin A1                  | Inhibits phospholipase A2 and has anti-inflammatory activity. |
Table 5. Cont.

| Code  | Name                          | Function                                                                 |
|-------|-------------------------------|--------------------------------------------------------------------------|
| CH    | DDWoR and MD                  |                                                                          |
| ANXA2 | Annexin A2                    | Functions as an autocrine factor which heightens osteoclast formation and bone resorption. |
| ASPN  | Asporin                       | Regulate chondrogenesis by inhibiting transforming growth factor-beta 1-induced gene expression in cartilage. May induce collagen mineralization. |
| BGN   | Biglycan                      | Plays a role in bone growth, muscle development and regeneration, and collagen fibril assembly in multiple tissues. This protein may also regulate inflammation and innate immunity. |
| CILP  | Cartilage Intermediate Layer Protein | This protein is present in the cartilage intermediate layer protein (CILP), which increases in early osteoarthrosis cartilage. |
| CLU   | Clusterin                     | Under stress conditions can be found in the cell cytosol. May be involved in cell death, tumor progression, and neurodegenerative disorders |
| COMP  | Thrombospondin-5              | Present in rheumatoid arthritis, is a noncollagenous extracellular matrix protein. |
| DCN   | Decorin                       | Has a stimulatory effect on autophagy and inflammation and an inhibitory effect on angiogenesis and tumorigenesis. |
| FMOD  | Fibromodulin                  | May also regulate TGF-beta activities by sequestering TGF-beta into the extracellular matrix. |
| FN1   | Fibronectin 1                 | Fibronectin is involved in cell adhesion and migration processes including embryogenesis, wound healing, blood coagulation, host defense. |
| IGHG1 | Immunoglobulin Heavy Constant Gamma 1 (G1m Marker) | Involved in pathways of Interleukin-4 and 13 signaling and IL4-mediated signaling events. |

Table 6. Gene code, protein name and function for each sample of TMJ discs.

| Code  | Name                          | Function                                                                 |
|-------|-------------------------------|--------------------------------------------------------------------------|
| DDWoR | Alpha-1-Microglobulin/Bikunin Precursor | Regulation of the inflammatory process. |
| MMP10 | Matrix Metallopeptidase 10    | Breakdown of extracellular matrix.                                      |
| MMP27 | Matrix Metallopeptidase 27    | Breakdown of extracellular matrix.                                      |
| MMP3  | Matrix Metallopeptidase 3     | Breakdown of extracellular matrix.                                      |
| PLA2G7| Phospholipase A2 Group VII    | Inflammatory and oxidative stress response.                            |
## Table 6. Cont.

### Disc Sample

| Code  | Name                          | Function                                                                 |
|-------|-------------------------------|--------------------------------------------------------------------------|
| **DDWoR** |                               |                                                                          |
| THADA | THADA Armadillo Repeat Containing | Apoptosis pathway.                                                         |
| THBS3 | Thrombospondin 3               | Matrix interactions.                                                      |
| **MD** |                               |                                                                          |
| AKAP13 | A-kinase anchor protein 13    | Regulation of apoptotic process.                                           |
| CCDC88A | Girdin                        | Vascular endothelial growth factor receptor 2 binding.                    |
| COL4A1 | Collagen alpha-1(IV) chain   | Extracellular matrix structural constituent.                               |
| ERAS  | GTPase ERas                   | Tumor-like growth properties of embryonic stem cells.                     |
| ERBIN | Erbin                         | Inhibits NOD2-dependent NF-kappa-B signaling and proinflammatory cytokine secretion. |
| PARP10 | Protein mono-ADP-ribosyltransferase PARP10 | Negative regulation of fibroblast proliferation. |
| PPFIA1 | Liprin-alpha-1                | Cell–matrix adhesion.                                                    |
| PPFIA2 | Liprin-alpha-2                | Cell–matrix adhesion.                                                    |
| PTPN7 | Tyrosine-protein phosphatase non-receptor type 7 | Regulation of T and B-lymphocyte development and signal transduction. |
| UPK3A | Uroplakin-3a                  | Epithelial cell differentiation.                                          |
| **CH** |                               |                                                                          |
| ACTN4 | Actinin Alpha 4               | Transcriptional coactivator.                                              |
| ADAM10 | ADAM Metallopeptidase Domain 10 | Responsible for the FasL ectodomain shedding. |
| COQ8B | Coenzyme Q8B                  | Biosynthesis of coenzyme Q.                                               |
| HPX   | Hemopexin                     | Protect cells from oxidative stress.                                      |
| HSPA1A | Heat Shock Protein Family A (Hsp70) Member 1A | Protection of the proteome from stress.                                  |
| NEK10 | NIMA Related Kinase 10        | Cellular response to UV irradiation.                                      |
| PDLIM4 | PDZ And LIM Domain 4          | Involved in bone development.                                             |
| SERPINH1 | Serpin Family H Member 1     | Chaperone in the biosynthetic pathway of collagen.                        |
| TTR   | Transthyretin                  | Thyroid hormone-binding protein.                                          |
| COL1A2 | Collagen Type I Alpha 2 Chain | Fibril-forming collagen abundant in bone.                                 |
| PRG4  | Proteoglycan 4                | This protein contains both chondroitin sulfate and keratan sulfate glycosaminoglycans. |
| PTPN13 | Protein Tyrosine Phosphatase Non-Receptor Type 13 | Regulates negatively FasL induced apoptosis. |
| **DDWoR and MD** |                               |                                                                          |
| C4A   | Complement C4A                | Antimicrobial peptide and a mediator of local inflammation.               |
| C4B   | Complement C4B                | Mediator of local inflammation.                                           |
| C4B_2 | Complement Component 4B       | Mediator of local inflammatory process.                                   |
| SEMA4F | Semaphorin 4F                 | Plays a role in neural development.                                       |
| **DDWoR and CH** |                               |                                                                          |
| ACAN  | Aggrecan                      | Part of the extracellular matrix that withstands compression in cartilage. |
| COL1A1 | Collagen Type I Alpha 1 Chain | Collagen component.                                                      |
### Table 6. Cont.

| Code   | Name                                      | Function                                                                 |
|--------|-------------------------------------------|--------------------------------------------------------------------------|
|        | **DDWoR and CH**                          |                                                                          |
| COL4A6 | Collagen Type IV Alpha 6 Chain            | Major structural component of basement membranes.                        |
| HSPA2  | Heat Shock Protein Family A (Hsp70) Member 2 | Protection of the proteome from stress.                                  |
| POSTN  | Periostin                                  | Extracellular matrix protein that functions in tissue development and regeneration, including wound healing. |
|        | **MD and CH**                             |                                                                          |
| KRT6A  | Keratin 6A                                | Epidermis-specific type I keratin involved in wound healing.             |
|        | **DDWoR, MD and CH**                      |                                                                          |
| ANXA1  | Annexin A1                                | Anti-inflammatory activity.                                              |
| ANXA2  | Annexin A2                                | Heightens osteoclast formation and bone resorption.                     |
| ANXA2P2| Annexin A2 Pseudogene 2                   | May be involved in heat-stress response.                                 |
| APCS   | Amyloid P Component                       | Is involved in dealing with apoptotic cells in vivo.                    |
| ASPN   | Asporin                                    | Regulates chondrogenesis by inhibiting transforming growth factor-beta 1-induced gene expression in cartilage |
| BGN    | Biglycan                                   | Plays a role in bone growth, and collagen fibril assembly in multiple tissues. This protein may also regulate inflammation and innate immunity. |
| C3     | Complement C3                             | Modulates inflammation and possesses antimicrobial activity.            |
| CILP   | Cartilage Intermediate Layer Protein      | Increases in early osteoarthrosis cartilage.                            |
| COL12A1| Collagen Type XII Alpha 1 Chain           | Type XII collagen.                                                      |
| COL14A1| Collagen Type XIV Alpha 1 Chain           | Type XIV collagen.                                                      |
| COL6A1 | Collagen Type VI Alpha 1 Chain            | Collagen VI.                                                            |
| COL6A2 | Collagen Type VI Alpha 2 Chain            | Type VI collagen.                                                       |
| COL6A3 | Collagen Type VI Alpha 3 Chain            | Type VI collagen.                                                       |
| COMP   | Cartilage Oligomeric Matrix Protein       | Degradation of the extracellular matrix.                                |
| ENO1   | Enolase 1                                 | Tumor suppressor.                                                       |
| ENO2   | Enolase 2                                 | Found in mature neurons and cells of neuronal origin.                   |
| ENO3   | Enolase 3                                 | Plays a role in muscle development and regeneration.                    |
| FN1    | Fibronectin 1                             | Involved in wound healing, blood coagulation, host defense.             |
| KRT7   | Keratin 7                                 | Co-expressed during differentiation of simple and stratified epithelial tissues. |
| LUM    | Lumican                                   | May regulate collagen fibril organization, epithelial cell migration and tissue repair. |
| MFAP4  | Microfibril Associated Protein 4          | Extracellular matrix protein which is involved in cell adhesion or intercellular interactions. |
| MFGE8  | Milk Fat Globule EGF And Factor V/VIII Domain Containing | Promotes phagocytosis of apoptotic cells. This protein has also been implicated in wound healing, autoimmune disease, and cancer. |
| OGN    | Osteoglycin                               | Induces ectopic bone formation in conjunction with transforming growth factor beta and may regulate osteoblast differentiation. |
Table 6. Cont.

| Code          | Name                          | Function                                                                 |
|---------------|-------------------------------|--------------------------------------------------------------------------|
| MD and CH     |                               |                                                                          |
| DDWoR, MD and CH |                 |                                                                          |
| SOD3          | Superoxide Dismutase 3        | Antioxidant enzymes that protect tissues from oxidative stress.           |
| TGFB1         | Transforming Growth Factor Beta Induced | May be involved in endochondrial bone formation in cartilage.              |
| TNC           | Tenascin C                    | Modulation of inflammatory cytokine.                                     |
| TNXB          | Tenascin XB                   | Accelerates collagen fibril formation.                                   |
| VCAN          | Versican                      | A large chondroitin sulfate proteoglycan and is a major component of the extracellular matrix. |
| VIM           | Vimentin                      | Involved in the stabilization of type I collagen mRNAs for CO1A1 and CO1A2. |

The disc sample presented the following protein functions for each group (Table 6): the DDWoR group expressed proteins involved in inflammatory process, neurogenesis, cartilage formation, extracellular matrix degradation, oxidative stress and apoptosis. The MD group presented proteins related to apoptosis, vascular growth, inflammatory inhibitors, immunologic factors and epithelial growth, and the CH group showed protein expression implicated in apoptosis, apoptosis inhibition, oxidative stress, bone formation, chondroïtin, bone and cartilage formation. The group with DDWoR and MD samples had proteins involved in inflammatory process; the group with DDWoR and CH samples showed proteins with collagen formation and wound healing functions; the group with MD and CH was involved in wound healing; and the group containing DDWoR, MD and CH samples was involved with inflammatory cascade modulation, osteoclastogenesis, chondrogenesis, apoptosis, bone formation, vascular and tissue repair, antioxidative activity.

There were proteins identified in both synovial fluid and TMJ disc samples, however, some of them in different pathology groups (Table 7).

Table 7. Name and function of expressed proteins in common between synovial fluid and TMJ disc sample, and the groups in each protein was expressed.

| Name                              | Function                                                                 | Disc                | Synovial Fluid       |
|-----------------------------------|--------------------------------------------------------------------------|---------------------|----------------------|
| Amyloid P Component, Serum        | Is involved in dealing with apoptotic cells in vivo.                     | DDWoR, MD and CH    | DDWoR                |
| Annexin A1                        | Anti-inflammatory activity.                                               | DDWoR, MD and CH    | DDWoR and MD         |
| Annexin A2                        | Heightens osteoclast formation and bone resorption.                      | DDWoR, MD and CH    | DDWoR and MD         |
| Asporin                           | Regulates chondrogenesis.                                                | DDWoR, MD and CH    | DDWoR and MD         |
| Biglycan                           | Plays a role in bone growth, and collagen fibril assembly in multiple tissues. | DDWoR, MD and CH    | DDWoR and MD         |
| Cartilage Intermediate Layer Protein | Increases in early osteoarthrosis cartilage.                          | DDWoR, MD and CH    | DDWoR and MD         |
| Complement C4A                     | Antimicrobial peptide and a mediator of local inflammation.             | DDWoR and MD        | MD                   |
| Enolase 2                          | Found in mature neurons and cells of neuronal origin.                   | DDWoR, MD and CH    | DDWoR, MD and CH     |
Table 7. Cont.

| Name                      | Function                                                                 | Disc                      | Synovial Fluid                  |
|---------------------------|--------------------------------------------------------------------------|---------------------------|---------------------------------|
| Enolase 3                 | Play a role in muscle development and regeneration.                      | DDWoR, MD and CH          | DDWoR, MD and CH                |
| Fibronectin 1             | Involved in wound healing, blood coagulation, host defense.              | DDWoR, MD and CH          | DDWoR and MD                    |
| Hemopexin                 | Protect cells from oxidative stress.                                     | CH                        | MD                              |
| Lumican                   | May regulate collagen fibril organization, epithelial cell migration and tissue repair. | DDWoR, MD and CH          | DDWoR and MD                    |
| Osteoglycin               | Regulate osteoblast differentiation.                                      | DDWoR, MD and CH          | DDWoR and MD                    |
| Serpin Family H Member 1 | Chaperones in the biosynthetic pathway of collagen.                      | CH                        | MD                              |
| Superoxide Dismutase 3   | Antioxidant enzymes that protect tissues from oxidative stress.          | DDWoR, MD and CH          | DDWoR and MD                    |
| Tenascin XB               | Modulation of inflammatory cytokine.                                     | DDWoR, MD and CH          | DDWoR and MD                    |
| Transforming Growth Factor Beta Induced | May be involved in endochondral bone formation in cartilage. | DDWoR, MD and CH          | DDWoR and MD                    |
| Versican                  | A large chondroitin sulfate proteoglycan and is a major component of the extracellular matrix. | DDWoR, MD and CH          | DDWoR and MD                    |

Different types of collagen were identified in discs of the MD group, CH group, DDWoR and CH group, and in the group with all pathologies together (DDWoR, MD and CH). Besides the known collagen type I present in TMJ discs, collagen type IV, VI, XII and XIV were also identified (Table 8).

Table 8. Types of collagen identified in each TMJ disc group.

| Type of Collagen Identified in Each Group | DDWoR and MD | CH | DDWoR and MD | DDWoR and CH | MD and CH | DDWoR, MD and CH |
|------------------------------------------|--------------|----|--------------|--------------|-----------|------------------|
| COL4A1 Collagen Type IV Alpha 1 Chain    | COL1A2 Collagen Type I Alpha 2 Chain | COL1A1 Collagen Type I Alpha 1 Chain | COL12A1 Collagen Type XII Alpha 1 Chain |
| COL4A6 Collagen Type IV Alpha 6 Chain    | COL14A1 Collagen Type XIV Alpha 1 Chain | COL6A1 Collagen Type VI Alpha 1 Chain |
| COL6A2 Collagen Type VI Alpha 2 Chain    | COL6A3 Collagen Type VI Alpha 3 Chain |

All shared and group-specific proteins are indicated in a Venn diagram for the synovial fluid (Figure 1) and disc samples (Figure 2).
Different types of collagen were identified in discs of the MD group, CH group, DDoW, and CH group, and in the group with all pathologies together (DDDoW, MD, and CH). Besides the known collagen type I present in TMJ discs, collagen type IV, VI, XII, and XIV were also identified (Table 8).

All shared and group-specific proteins are indicated in a Venn diagram for the synovial fluid (Figure 1) and disc samples (Figure 2).

![Venn diagram for synovial fluid](image1)

**Figure 1.** Venn diagram for synovial fluid: group 1—DDDoW, group 2—MD, group 3—CH.

![Venn diagram for TMJ disc](image2)

**Figure 2.** Venn diagram for the TMJ disc: group 1—DDDoW, group 2—MD, group 3—CH.

The interactions between the proteins were analyzed with Genemania (https://genemania.org—accessed on 5 September 2020), and its genetic network pointed out distinct protein cascades that might be modulating each pathology through the synovial fluid and disc samples. The physical and genetic interactions, co-expression and pathway of the proteins are shown in Figures 3 and 4.
samples. The physical and genetic interactions, co-expression and pathway of the proteins are shown in Figures 3 and 4.

The main proteins with important functions and networks that were identified in the synovial fluid sample were analyzed for each group (Figure 3). A brief description of these findings are: in the DDWoR group (Figure 3A) alpha-2-macroglobulin (A2M) involved in inflammatory process, amyloid P component (APCS) involved with apoptosis and complement factor H (CFH) that modulates inflammatory cascade were highlighted in the Genemania interaction figure; in the MD group (Figure 3B), hemopexin (HPX) involved in protection against oxidative stress was present; in the CH group (Figure 3C), alcohol dehydrogenase subunit alpha (ADH1) that is responsible for alcohol degradation and interacts with growth hormone receptor (GHR) was present. In the group of DDWoR and MD (Figure 3D), annexin A1 (ANXA1), decorin (DCN), and immunoglobulin heavy constant gamma 1 (IGHG1) involved in inflammatory process, annexin A2 (ANXA2) involved with bone resorption, asporin (ASPN), biglycan (BGN), cartilage intermediate layer protein (CILP), osteoglycin (OGN), transforming growth factor beta induced (TGFBI) involved in bone and cartilage formation, fibronectin 1 (FN1), lumican (LUM) and tenascin XB (TNXB) involved in tissue repair, and neurofilament medium (NEFM) and thrombospondin 4 (THBS4) involved in neuropathic pain were included in the net.

The DDWoR and CH group, and MD and CH group had no protein to be analyzed. The group with the three pathologies (DDWoR, MD and CH) showed an interaction of enolase 2 (ENO2) and 3 (ENO3), involved in muscle regeneration (Figure 3E).

**Figure 3.** Gene interactions between the main functional proteins of synovial fluid. (A) showing the gene interactions of the DDWoR group. (B) showing the gene interactions of the MD group. (C) showing the gene interactions of the CH group. (D) showing the gene interactions of the DDWoR and MD group. (E) showing the gene interactions of the DDWoR, MD and CH group.
Figure 3. Gene interactions between the main functional proteins of synovial fluid. (A) showing the gene interactions of the DDWoR group. (B) showing the gene interactions of the MD group. (C) showing the gene interactions of the CH group. (D) showing the gene interactions of the DDWoR and MD group. (E) showing the gene interactions of the DDWoR, MD and CH group.

Figure 4. Cont.
The main proteins with important functions and networks that were identified in the synovial fluid sample were analyzed for each group (Figure 3). A brief description of these findings are: in the DDWoR group (Figure 3A) alpha-2-macroglobulin (A2M) involved in inflammatory process, amyloid P component (APCS) involved with apoptosis and complement factor H (CFH) that modulates inflammatory cascade were highlighted in the Genemania interaction figure; in the MD group (Figure 3B), hemopexin (HPX) involved in protection against oxidative stress was present; in the CH group (Figure 3C), alcohol dehydrogenase subunit alpha (ADH1) that is responsible for alcohol degradation and interacts with growth hormone receptor (GHR) was present. In the group of DDWoR and MD (Figure 3D), annexin A1 (ANXA1), decorin (DCN), and immunoglobulin heavy constant gamma 1 (IGHG1) involved in inflammatory process, annexin A2 (ANXA2) involved with bone resorption, asporin (ASPN), biglycan (BGN), cartilage intermediate layer protein (CILP), osteoglycin (OGN), transforming growth factor beta induced (TGFBI) involved in bone and cartilage formation, fibronectin 1 (FN1), lumican (LUM) and tenascin XB (TNXB) involved in tissue repair and neurofilament medium (NEFM) and thrombospondin 4 (THBS4) involved in neuropathic pain were included in the net. The DDWoR and CH group, and MD and CH group had no protein to be analyzed. The group with the three pathologies (DDWoR, MD and CH) showed an interaction of enolase 2 (ENO2) and 3 (ENO3), involved in muscle regeneration (Figure 3E).

The disc sample presented the following protein interactions in Genemania (Figure 4): group DDWoR (Figure 4A) presented mainly the matrix metalloproteinase protein (MMP) family (1,2,5,6,8,10,13,15,16), integrin subunit alpha 6 (ITGA6) and phospholipase A2 group VII (PLA2G7) that are involved in inflammatory cascade. Additionally, thrombospondin 3 (THBS3) and 4 (THBS4) involved in tissue remodeling, and THADA armadillo repeat containing (THADA) involved in apoptosis were present. In the MD group (Figure 4B), A-kinase anchor protein 13 (AKAP13), Erbin (ERBIN) and uroplakin-3a (UPK3A) involved in apoptosis, collagen alpha-1(IV) chain (COL4A1) and GTPase Eras (ERAS) involved in disc matrix constitution, and liprin-alpha-1 (PPFIA1) and (PPFIA2) 2 responsible for cell interactions were identified in the Genemania network. In the CH group (Figure 4C), the present proteins were ADAM metallopeptidase domain 10 (ADAM10), that regulates apoptosis, collagen type I alpha 2 chain (COL1A2) and serpin family H member 1 (SERPINH1) involved in collagen formation, actinin alpha 4 (ACTN4), PDZ Additionally, LIM domain 4 (PDLIM4), transhyretin (TTR) and protein tyrosine phosphatase non-receptor type 13

![Figure 4. Gene interactions between the main functional proteins of the TMJ disc. (A) showing the gene interactions of the DDWoR group. (B) showing the gene interactions of the MD group. (C) showing the gene interactions of the CH group. (D) showing the gene interactions of the MD and CH group. (E) showing the gene interactions of the DDWoR and CH group. (F) showing the gene interactions of the DDWoR and CH group. (G) showing the gene interactions of the DDWoR, MD and CH group.](image-url)
(PTPN13) involved in apoptosis, hormone modulation and bone formation. In the group of DDWoR and MD (Figure 4D), the complement C4A (C4A) and complement C4B (C4B) proteins that mediates the inflammatory process were identified. In the DDWoR and CH group (Figure 4E), mainly the proteins aggrecan (ACAN), collagen type I alpha 1 chain (COL1A1) and collagen type IV alpha 6 chain (COL4A6) that constitutes disc matrix, and periostin (POSTN) involved in wound healing were identified. In the MD and CH group (Figure 4F), keratin 6A (KRT6A) involved in wound healing was identified. Additionally, in the group with all three pathologies (DDWoR, MD and CH) the proteins that interacted were annexin A1 (ANXA1), complement C3 (C3) and tenasin C (TNC) involved in inflammatory cascade modulation, annexin A2 (ANXA2) and transforming growth factor beta induced (TGFBI) involved in osteoclastogenesis, asporin (ASPN), biglycan (BGN), collagen type VI alpha 1 chain (COL6A1), osteoglycin (OGN) and vimentin (VIM) involved in chondrogenesis and osteogenesis, amyloid P component (APCS) and complement C3 (C3) in apoptosis and lumican (LUM) involved in tissue repair (Figure 4G).

4. Discussion

The different types of TMD may jeopardize patients’ quality of life, masticatory function and have a great impact on health expenses. The identification of its multifactorial etiological components will enhance the employment of specific treatments, diminishing the hazard it causes in the TMJ. Therefore, the identification of the proteins expressed on each pathology group of this study (DDWoR, MD, and CH) might elucidate the cascades involved in the progression and severity of each TMD, leading to an assertive handling of TMD.

A total of 225 proteins were identified in the synovial fluid sample, and 379 in the TMJ disc sample (Table 2). It is important to highlight that the synovial fluid sample is very complex to obtain, therefore some proteins might not have been identified due to the technique that advocates the dilution of the synovial fluid. Nevertheless, the sample was collected according to worldwide employed standard methods previously described by other research groups [21,25]. Additionally, even though few proteins’ expression might not have been observed, the expression of new proteins were identified for each pathology group, which enriches the global analysis of this study.

In our analysis, we found that all proteins expressed in the DDWoR group (synovial fluid and disc sample) (Tables 2 and 3) presented many proteins related to inflammatory process (MMP-3, -10, -27 in the disc sample) and apoptosis (mitogen-activated protein kinase 7—MAP3K7) and THADA in synovial fluid). Only the MMP-3 protein was previously associated with TMD [26,27]. These are proteins that highly impact the degeneration process in the TMJ of patients with DDWoR [26,28]. In the MD group, ERBIN protein was found in the disc sample, and it modulates TGFB, which was previously associated with TMJ degeneration [29]. Additionally, unprecedented proteins were seen in the synovial fluid associated with apoptosis (aldehyde dehydrogenase 1 family member L—ALDH1L1) and protection against oxidative stress (HPX), which probably helps diminish the mechanical overload consequences of the dislocation in the TMJ. Regarding CH proteins in the synovial fluid sample, ADH1 catalyzes the oxidation of alcohols to aldehydes, but as seen in Genemania (Figure 3C), it interacts with GHR, which might be involved with the condylar overgrowth. In a previous study, GHR has been injected in rabbits’ TMJ to increase cartilage thickness [30], but it has not been studied as a possible etiology of condylar overgrowth yet.

Additionally, we also found a set of proteins to be common in both synovial fluid and disc samples (Table 4) in the groups DDWoR (chromodomain-helicase-DNA-binding protein 8 and myosin light chain 6B), MD (filamin A and liprin-alpha-1), and in the three groups (enolase 1, 2, 3, myosin heavy chain 16, ribosomal protein L7 like 1 and component of the shield in complex). These proteins were involved in cell matrix adhesion, cellular motor protein, reorganization of cytoskeleton, muscle development and regeneration. Additionally, another group of proteins were identified in both synovial fluid and disc
samples (Table 7), being prevalent in all groups of disc samples. In the DDWoR and MD groups of synovial fluid samples, proteins implicated in apoptosis, inflammatory process, bone formation and resorption, chondrogenesis, wound healing, tissue repair and protection against oxidative stress were found. CH disc samples and MD synovial fluid samples presented, as common proteins, HPX (protection against oxidative stress) and SERPINC1 (biosynthetic pathway of collagen).

LUM is associated with the regulation of collagen fibers and with cell migration. In this study, LUM was present in all disc samples, and it has been pointed out to be elevated when the disc is under stress, as it enhances tissue repair [31]. Ulmner [32] reported that higher levels of LUM in synovial tissue might diminish TMD surgical success. On the other hand, TNC was present in all disc samples and in DDWoR and MD synovial fluid sample, being an important protein in wound healing [33].

Temporomandibular joint discs are fibrocartilaginous discs composed mainly by collagen, glycosaminoglycan and proteoglycans [34]. Studies in human adults and fetuses showed the expression of mainly collagen type I and III in TMJ discs, with type I collagen observed in the posterior band of the articular disc and collagen type III on the inferior surface of the articular disc [35,36]. Moreover, collagen type II synthesis was expressed on the external layer of the TMJ disc [37]. In this study, collagen type IV was identified in MD and CH samples (Table 8), and a previous study observed the presence of collagen type IV in the middle part of fetuses’ TMJ disc, indicating the development of blood vessels [38]. The TMJ disc is an avascular tissue, although under stress it may undergo metaplasia, forming a vascularized fibrous tissue. Collagen type VII was present in all samples, and along with collagen type IV, it has chondroprotective effects against inflammation [39].

Collagen type XII and XIV were present in the disc samples of this study, which have never been identified in this region before in humans. A study identified collagen type XII only in bovine disc samples, which helps maintain collagen type I integrity [40]. Nevertheless, collagen type XIV was also observed in all TMJ disc samples, and it plays an essential structural role in the integrity of collagen type I, mechanical properties, organization, and shape of articular cartilage, which has never been described in the TMJ disc before [41]. This is important information to understand the composition’s strength and weakness of the TMJ disc.

5. Conclusions

In conclusion, many proteins were identified for the first time in the TMJ disc and synovial fluid of the groups DDWoR, MD and CH, leading to the enlightenment of each pathology’s etiology, modulation and progression. Further studies with a greater sample are necessary to evaluate other proteins that might be present in these pathologies as well.
Data Availability Statement: Data is data contained within the article.

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