Paulo Santoro Belangero

AVALIAÇÃO DA EXPRESSÃO GÊNICA DA CÁPSULA ARTICULAR NA INSTABILIDADE ANTERIOR TRAUMÁTICA DO OMBRO

Tese apresentada à Universidade Federal de São Paulo – Escola Paulista de Medicina, para obtenção do Título de Doutor em Ciências.

São Paulo

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ORIENTADOR: Prof. Dr. Moisés Cohen

CO-ORIENTADORES: Prof. Dr. Benno Ejnisman
Prof.ª Dr.ª Mariana Ferreira Leal

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Coordenador do Curso de Pós-Graduação em Cirurgia Translacional:

Prof. Dr. Miguel Sabino Neto

Universidade Federal de São Paulo

Reitora: Prof.ª Dr.ª Soraya Soubhi Smaili

Vice-reitora: Prof.ª Dr.ª Valéria Petri

Chefe de Gabinete: Prof.ª Dr.ª Maria José da Silva Fernandes

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ORIENTADOR

Prof. Dr. Moisés Cohen

Professor Titular do Departamento de Ortopedia e Traumatologia

Universidade Federal de São Paulo - Escola Paulista de Medicina

CO-ORIENTADORES

Prof. Dr. Benno Ejnisman

Professor Adjunto do Departamento de Ortopedia e Traumatologia

Universidade Federal de São Paulo - Escola Paulista de Medicina

Prof.ª Dr.ª Mariana Ferreira Leal

Professora Afiliada do Departamento de Ortopedia e Traumatologia

Professora Substituta do Departamento de Morfologia e Genética

Universidade Federal de São Paulo - Escola Paulista de Medicina

EQUIPE DE PESQUISA

Disciplina de Medicina Esportiva e Atividade Física
Departamento de Ortopedia e Traumatologia
Escola Paulista de Medicina – Universidade Federal de São Paulo

- Prof. Dr. Moisés Cohen – Professor Titular e Chefe de Departamento
- Prof. Dr. Benno Ejnisman – Professor Adjunto e Chefe da Disciplina
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- Carina Cohen – Doutoranda
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Disciplina de Genética
Departamento de Morfologia e Genética
Escola Paulista de Medicina – Universidade Federal de São Paulo

- Prof.ª Dr.ª Marília de Arruda Cardoso Smith – Professora Titular e Vice-Chefe da Disciplina
- Prof.ª Dr.ª Sintia Iole Belangero – Professora Adjunta e Coordenadora do Programa de Pós-Graduação em Biologia Estrutural e Funcional

Paulo Santoro Belangero

AVALIAÇÃO DA EXPRESSÃO GÊNICA DA CÁPSULA ARTICULAR NA INSTABILIDADE ANTERIOR TRAUMÁTICA DO OMBRO

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DEDICATÓRIA

Aos meus três amores,
Sintia, Laura e Beatriz,
por fazerem da minha vida um sonho.

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“A dúvida é o princípio da sabedoria.”

Aristóteles

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LISTA DE ABREVIATURAS E SÍMBOLOS

Lista de abreviaturas

AGU	Articulação Glenoumeral
AI	Anteroinferior
AS	Anterossuperior
CLGUI	Complexo Ligamentar Glenoumeral Inferior
COL1A1	Colágeno, Tipo I, Alfa 1
COL1A2	Colágeno, Tipo I, Alfa 2
COL3A1	Colágeno, Tipo III, Alfa 1
COL5A1	Colágeno, Tipo V, Alfa 1
COL5A2	Colágeno, Tipo V, Alfa 2
COL5A3	Colágeno, Tipo V, Alfa 3
COMP	Proteínas Oligoméricas Da Matriz Da Cartilagem
EDS	Síndrome Ehlers-Danlos
FN	Fibronectina
FN1	Fibronectina 1
IATO	Instabilidade Anterior Traumática Do Ombro
IR	Intervalo Rotador
LCA	Ligamento Cruzado Anterior
LCU	Ligamento Coracoumeral
LGUM	Ligamento Glenoumeral Médio
LGUS	Ligamento Glenoumeral Superior
LH	Lisil Hidroxilase
LOX	Lisil Oxidase
MEC	Matriz Extra Celular
mRNA	RNA Mensageiro
N	Tamanho Amostral
OI	Osteogênese Imperfeita
P	Posterior
PLOD1	Pró-colágeno-Lisina, 2-Oxoglutarate 5-Dioxygenase 1
PLOD2	Pró-colágeno-Lisina, 2-Oxoglutarate 5-Dioxygenase 2
PLOD3	Pró-colágeno-Lisina, 2-Oxoglutarate 5-Dioxygenase 3
qRT-PCR	Reação em Cadeia da Polimerase Quantitativa via Transcriptase Reversa
TN	Tenascina
TGFB1	Fator de crescimento transformante, Beta 1
TGFBR1	Transforming Growth Factor, Beta Receptor 1
TNC	Tenascina C
TNR	Tenascina R
TNXA	Tenascina XA
TNXB	Tenascina XB

Lista de símbolos

kb	kilobase
kDa	kiloDalton

RESUMO

O ombro é a articulação humana mais suscetível a luxações, com incidência estimada de 8,2-23,9 casos por 100.000 indivíduos por ano. O estudo das alterações moleculares relacionadas a instabilidade do ombro pode favorecer a compreensão da fisiopatologia da doença. Nós hipotetizamos que a expressão de genes relacionados ao colágeno, à regulação de suas fibrilas e genes relacionados à matriz extracelular possam estar envolvidos com a instabilidade anterior traumática do ombro e seus aspectos clínicos, tais como gravidade e tempo de doença. Assim, nossos objetivos foram avaliar a expressão desses genes na cápsula articular do ombro de pacientes com instabilidade anterior traumática e de controles, relacionando-os com aspectos clínicos e etiológicos. Para isso, estudamos 31 pacientes com instabilidade anterior traumática do ombro e oito indivíduos controle (portadores de luxação acromioclavicular). Três regiões da cápsula foram coletadas: anteroinferior, anterossuperior, posterior. A expressão dos genes foi quantificada por meio de qRT-PCR. Encontramos alteração da expressão de genes dos três grupos: a) codificadores de colágeno; b) genes reguladores da expressão e da estrutura de fibrilas do colágeno e c) genes da matriz extracelular, nas três regiões capsulares estudadas de pacientes com instabilidade anterior traumática do ombro, incluindo a região posterior. As diferenças foram mais relevantes na região anteroinferior, região macroscopicamente afetada, entretanto a presença de alterações na região posterior representou o dado positivo mais surpreendente. Na comparação entre casos e controles, os genes *COL1A1, COL1A2, COL3A1, COL5A1* (genes codificadores de colágeno), *TGFB1, TGFBR1, PLOD2, COMP* (genes relacionados à formação das fibrilas colágenas -*crosslink*) e *FN1, TNC e TNXB* (genes relacionados a matriz extracelular) mostraram-se diferencialmente expressos e, dessa forma, podem ser relevantes para a fisiopatologia da instabilidade traumática do ombro. Em relação ao tempo de doença, os genes *COL1A1, TGFB1, TGFBR1, PLOD2, FN1 e TNXB* mostraram-se diferentes e, assim, podem ser estar relacionados à evolução da doença. Quanto ao número de episódios, *TGFBR1, PLOD2, LOX, FN1 COL1A1, COL3A1, COL5A1,* mostraram-se diferentes e também podem ser estar associados à gravidade. Nossos achados demonstram que existe uma alteração na expressão de genes envolvidos na estrutura e/ou manutenção da cápsula glenoumeral de indivíduos com instabilidade anterior traumática. Essas alterações ocorrem mesmo após um único

episódio de luxação e estão relacionadas a situações clínicas que representam gravidade da doença, como número de episódios de luxação e tempo de duração da enfermidade. Este foi o primeiro estudo de expressão gênica na cápsula articular do ombro e nossos dados contribuem para o entendimento dos mecanismos envolvidos na fisiopatologia da doença que, futuramente, poderão auxiliar no desenvolvimento de novas estratégias de diagnóstico ou até de tratamento pela identificação de novos alvos terapêuticos.

ABSTRACT

The shoulder is the human joint most susceptible to dislocations, with an estimated incidence of 8.2 to 23.9 cases per 100,000 individuals per year. Molecular studies related to shoulder instability could promote a better understanding of the pathophysiology of the disease. We hypothesize that the expression of genes related to collagen, regulating their fibrils and extracellular matrix-related genes might be involved in traumatic anterior shoulder instability and clinical aspects, such as severity and duration of disease. Thus, our objectives were to assess the gene expression in the shoulder joint capsule of patients with traumatic anterior instability and controls, relating them to clinical and etiological aspects. We studied 31 patients with traumatic anterior instability of the shoulder and eight control subjects (patients with acromioclavicular dislocation). Three regions of the capsule were collected: anterior, anterosuperior and posterior. The gene expression was quantified by qRT-PCR. We found alteration of gene expression of three groups: a) collagen encoders; b) regulating gene expression and fibril structure of collagen and c) genes of extracellular matrix; in the three capsular regions studied in the patients with traumatic anterior shoulder instability, including posterior region. The differences were most significant in the anterior region, macroscopically affected region, however the presence of alterations in the posterior region represented the most surprising positive result. Comparing cases and controls, *COL1A1, COL1A2, COL3A1, COL5A1* (collagen genes), *TGFB1, TGFBR1, PLOD2, COMP* (genes related to the formation of collagen fibrils -crosslink) and *FN1, TNC* and *TNXB* (related genes extracellular matrix) shown to be differentially expressed and, therefore, may be relevant to the pathophysiology of traumatic shoulder instability. Regarding the duration of disease, *COL1A1, TGFB1, TGFBR1, PLOD2, FN1* and *TNXB* were different and thus may be related to disease progression. Regarding the number of episodes, *TGFBR1*, *PLOD2, LOX, FN1 COL1A1, COL3A1, COL5A1*, were different and can also be associated with gravity. Our findings demonstrate that gene expression is involved to the structure and / or maintenance of the glenohumeral capsule individuals with traumatic anterior instability. These changes occur even after a single episode of dislocation and are related to clinical situations that represent disease severity, as the number of episodes of dislocation and time duration of the disease. This was the first study of gene expression in the articular capsule of the shoulder and our data contribute to the understanding of the mechanisms involved in the pathophysiology of the disease that, in a future might help develop new strategies for diagnosis or even treatment by identifying new therapeutic targets.

INTRODUÇÃO

INTRODUÇÃO

A presente tese foi derivada do primeiro estudo multidisciplinar envolvendo a Disciplina de Genética do Departamento de Morfologia e Genética e a Disciplina de Medicina Esportiva e Atividade Física do Departamento de Ortopedia e Traumatologia, ambos da Universidade Federal de São Paulo. Essa parceria deu origem à linha de pesquisa intitulada “Aspectos genéticos e moleculares em lesões musculoesqueléticas” que tem por objetivo unir a ciência básica e clínica para aprofundar o conhecimento de diversas doenças ortopédicas.

Para o nosso conhecimento, os estudos que compõem essa tese de doutorado foram os primeiros a investigarem a expressão gênica em pacientes com instabilidade anterior traumática do ombro (IATO). Para comprovar nossas hipóteses, utilizamos duas principais abordagens: um estudo caso-controle e um estudo de medidas pareadas do mesmo indivíduo, o que exclui fatores genéticos interindividuais.

A presente tese será composta das seguintes seções: “Revisão da literatura” que incluirá uma exposição sobre os aspectos anatômicos e clínicos da instabilidade do ombro, bem como uma breve descrição de suas bases genéticas; “Objetivos” no qual abordaremos o objetivo geral e os específicos; “Artigos científicos” (quatro artigos científicos derivados dessa tese e já publicados pelo nosso grupo) em substituição aos itens ‘Casuística e Métodos’, ‘Resultados’ e ‘Discussão’ de uma tese tradicional, uma vez que cada um apresenta particularidades relacionadas a esses itens; “Conclusões gerais” do estudo e “Limitações” do trabalho.

Os dados gerados a partir dessa tese são originais e permitirão uma maior compressão dos processos biológicos subjacentes da instabilidade do ombro. Tais fatos podem ser úteis para o entendimento dos mecanismos envolvidos na fisiopatologia da doença e, no futuro, poderão servir de base para novas formas de diagnóstico e alvos terapêuticos.

OBJETIVOS

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1.1 Objetivo Geral

Identificar fatores genéticos relevantes para a fisiopatologia da instabilidade anterior traumática do ombro.

1.2 Objetivos específicos

1) Comparar a expressão dos genes *COL1A1, COL1A2, COL3A1, COL5A1, TGFB1 , TGFBR1 , LOX, PLOD1, PLOD2, COMP, TNXB, FN1* e *TNC* entre:
   a) indivíduos com IATO e controles em três diferentes regiões da cápsula da articulação glenoumeral [anteroinferior (AI), anterossuperior (AS) e posterior (P)];
   b) as regiões da cápsula supramencionadas em pacientes portadores de IATO.

2) Correlacionar o perfil de expressão gênica com os dados clínicos dos pacientes.

REVISÃO DA LITERATURA

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1.3 Estabilidade da articulação glenoumeral

O aspecto mais marcante da articulação glenoumeral (AGU) é a capacidade de estabilizar a cabeça umeral no centro da glenoide e permitir, ao mesmo tempo, uma enorme amplitude de movimento (Rockwood et al, 2009). Esse equilíbrio refinado é obtido pela combinação de mecanismos estáticos e dinâmicos presentes na AGU, que atuam em diferentes sentidos, com intensidade variável, mas que harmonicamente produzem um vetor de força resultante que mantém a cabeça umeral perfeitamente no centro da glenoide (Rockwood et al, 2009). Durante grande parte da movimentação da AGU, a cápsula e os ligamentos estão frouxos e a cabeça do úmero se move por aplicação de forças musculares. Nos arcos intermediários de movimento, os estabilizadores mais significantes são a pressão intra-articular e a integridade óssea do úmero e da glenoide. Nos movimentos em final de amplitude, a translação da cabeça umeral é limitada pelo complexo capsuloligamentar (Howell & Galinat, 1989; Rockwood et al, 2009; Drury et al, 2010; Moore et al, 2010).

As estruturas associadas à estabilização da AGU podem ser divididas em estáticas e dinâmicas e serão mais bem descritas a seguir.

1.3.1 Estabilizadores dinâmicos

Os estabilizadores dinâmicos correspondem à musculatura do ombro, são eles: o conjunto de músculos que forma o manguito rotador (subescapular, supraespinhal, redondo menor e infraespinhal, o cabo longo do bíceps (CBL) e o músculo deltoide. O efeito estabilizador é devido à tensão muscular passiva, à compressão das superfícies articulares (efeito concavidade-compressão) pela musculatura, e pelo efeito-barreira da contração da musculatura do ombro (Rockwood et al, 2009).

O primeiro efeito parece ser de pouca eficácia pelo resultado de diferentes estudos (Howell & Kraft, 1991; Motzkin et al, 1994; Campbell et al, 2014). No entanto, o musculo subescapular tem papel na estabilização anterior entre zero e 45^{o} de abdução. A perda da tensão passiva do manguito rotador (lesões dos músculos supraespinhal e do infraespinhal) também tem sido associada à instabilidade posterior (Ovesen & Nielsen, 1986; Campbell et al, 2014). Diminuição da espessura e da área de secção transversa do tendão do subescapular é mais frequentemente encontrado em pacientes com

luxações anteriores recorrentes (Ovesen & Sojbjerg, 1986; Tuoheti et al, 2005; Jaggi et al, 2012).

O efeito concavidade-compressão está relacionado à profundidade da glenoide e à magnitude das forças compressivas. Enquanto alguns estudos demonstram que a estimulação do manguito rotador não tem efeito sobre o efeito concavidade-compressão, outros demonstram que a lesão dessa musculatura do ombro predispõe à instabilidade (Howell & Kraft, 1991; Wuelker et al, 1994; Gombera & Sekiya, 2014; Holscher et al, 2016).

1.3.1.1 Manguito rotador

Os dados sobre o efeito da contração dinâmica e sobre a estabilidade da AGU não são consensuais. Alguns autores têm salientado a importância do músculo supraespinhal como estabilizador inferior, outros afirmam que tal músculo está mais envolvido na estabilidade anterior (Rockwood et al, 2009). Estudos com eletroneuromiografia mostram que tanto o músculo supraespinhal quanto o infraespinhal são necessários para a estabilização durante a elevação do ombro (Saha, 1971). O músculo supraespinhal e o do cabo longo do bíceps possuem um papel compensador em ombros instáveis (Kronberg et al, 1991; Illyes et al, 2009). Entretanto, a importância do manguito rotador como fator de estabilização da AGU não é um consenso na literatura científica (Howell & Kraft, 1991).

Estudos para esclarecer o papel de cada componente do manguito rotador na estabilidade da AGU mostraram que o músculo infraespinhal e o redondo menor eram mais eficientes para controlar a rotação externa e reduzir a tensão dos ligamentos (Mulcahey et al, 2015). Em um estudo semelhante, porém aplicando cargas supra-fisiológicas, outro grupo concluiu que o subescapular é de importância primária na estabilização anterior do ombro em abdução e rotação neutra e, menos importante na rotação externa, posição na qual os músculos posteriores do manguito rotador passam a ser mais importantes (McKernan et al, 1990). O “efeito-barreira” (também chamado de “efeito de amortecimento”) é reconhecido como o mecanismo pelo qual o músculo subescapular opõe-se à luxação anteroinferior da cabeça umeral. Lesões dessa musculatura afetam o mecanismo estabilizador anterior e posterior de translação da cabeça umeral (Bassett et al, 1990).

1.3.1.2 Bíceps

O cabo longo do bíceps tem efeito na compressão da cabeça umeral, demonstrado inclusive por estímulo elétrico durante artroscopia (Pagnani et al, 1996; Hwang et al, 2014; Hwang et al, 2015). O efeito é maior em rotação externa e mínima em rotação interna. Estudo em cadáver demonstrou que o efeito como estabilizador do bíceps e do manguito rotator são semelhantes em ombros estáveis. No entanto, quando há instabilidade, o bíceps torna-se mais importante que qualquer componente do manguito rotador (Itoi et al, 1994). Deve-se salientar que *in vivo*, esses músculos atuam conjuntamente, e, em lesões do manguito rotador, a contração do bíceps pode prevenir a migração superior da cabeça do úmero (Kido et al, 2000).

1.3.1.3 Deltoide

O músculo deltoide corresponde a 20% da massa muscular do ombro. Dessa forma, é possível que esse músculo tenha um papel importante na estabilização da AGU (Bassett et al, 1990). No entanto, alguns estudos sugerem que a contribuição estática desse músculo é pouco importante (Motzkin et al, 1994). Outros estudos demonstraram um papel efetivo do bíceps durante grande parte do arco de movimento do ombro (Michiels & Bodem, 1992)(Lee & An, 2002).

A interação entre estabilizadores estáticos e dinâmicos é fundamental para a estabilidade do ombro. A importância de cada um desses fatores em determinadas posições ou funções do ombro ainda é pouco conhecida. Os estabilizadores dinâmicos parecem ser mais importantes quando os movimentos do ombro são restritos em termos de amplitude, enquanto os estabilizadores estáticos parecem atuar mais ativamente em movimentos com maiores amplitudes. Tanto o manguito rotador e quanto a cápsula possuem um papel na estabilização anterior, mas o manguito atua principalmente na estabilização posterior (Rockwood et al, 2009).

O complexo cápsula-ligamentos detectam a posição, o movimento e o estiramento por meio de sensores (mecanorreceptores) em um processo denominado de propriocepção. Esses sensores estão presentes no ligamento coracoacromial, na bursa subacromial, na cápsula e no lábio da glenoide (Rockwood et al, 2009). Em modelos animais, foi demonstrado um arco reflexo a partir dos mecanorreceptores dentro da AGU em direção à musculatura do ombro (Guanche et al, 1995). Nesse modelo, a estimulação

de ramos anteriores e inferiores do nervo axilar causava contração do bíceps e do manguito rotador, enquanto que a estimulação do ramo posterior levava à contração do deltoide. Esses achados demonstram uma íntima relação entre os estabilizadores estáticos e dinâmicos.

1.3.2 Estabilizadores estáticos

1.3.2.1 Estrutura óssea

A maioria dos estudos sobre a contribuição óssea à estabilidade do ombro está focada na glenoide. A leve retroversão de 7^{o} em relação à escápula tem grande importância para a estabilidade da AGU (Saha, 1971). Aumento da anteversão induz o aumento da instabilidade anterior. Por outro lado, o aumento da retroversão leva ao aumento da translação posterior da cabeça umeral. Do ponto de vista clínico, observa-se que enquanto a instabilidade anterior não está associada com anteversão da glenoide, a instabilidade posterior aumenta com a retroversão (Randelli & Gambrioli, 1986; Kim et al, 2005). Os 30^{o} de retroversão da cabeça umeral são essenciais para o equilíbrio capsulo ligamentar e dos movimentos da AGU. A relação entre o diâmetro da glenoide e os diferentes diâmetros da cabeça umeral, medidos em vários planos reflete a instabilidade inerente da AGU. A relação entre essas medidas, o índice glenoumeral, varia de 0,86 (plano sagital) a 0,58 (plano transverso) e demonstram quão crítico é a relação, especialmente, no plano transverso (Rockwood et al, 2009).

A prevalência de defeitos ósseos da cabeça umeral e da glenoide são elevados em pacientes com instabilidade anterior recorrente (Hill & Sachs, 1940; Henry & Genung, 1982; Hovelius, 1987; Baudi et al, 2013; Garcia et al, 2015; Hovelius & Rahme, 2016). Após o primeiro episódio de luxação anterior, até 22% dos pacientes apresentam defeitos ósseos na glenoide (Garcia et al, 2015).

1.3.2.1.1 Escápula

Biomecanicamente, a AGU corresponde a um mecanismo de cadeia-fechada incluindo ossos, ligamentos e músculos que asseguram estabilidade contra translação excessiva, permitindo a obtenção das inúmeras posições necessárias aos movimentos do braço e da mão. Do ponto de vista cinemático, esse balanço mimetiza o conjunto entre uma bola e um soquete. Assim, a escápula tem múltiplos papéis na manutenção da estabilidade desse conjunto (Kibler & Sciascia, 2016).

O primeiro papel da escápula é manter a glenoide dinamicamente posicionada em três dimensões, mantendo o angulo glenoumeral em uma zona segura que minimize o cisalhamento glenoumeral, maximize a relação concavidade/compressão e diminua a ativação muscular (Warner et al, 1992; Lippitt et al, 1993; Happee & Van der Helm, 1995) (Figura 1). Estudos biomecânicos estimaram o valor do ângulo glenoumeral de aproximadamente 30^{o} (Happee & Van der Helm, 1995). Nesse ângulo, a tensão sobre os ligamentos e a atividade muscular são minimizadas e permitem as melhores condições de estabilidade da AGU. Nessa posição, todos os músculos do manguito rotador podem ser contraídos em linha reta para maximizar a relação concavidade/compressão da articulação. O resultado funcional das atividades musculares produzindo posicionamento dinâmico é denominado de ritmo escápulo-umeral, que resulta no movimento acoplado, síncrono do úmero e da escápula.

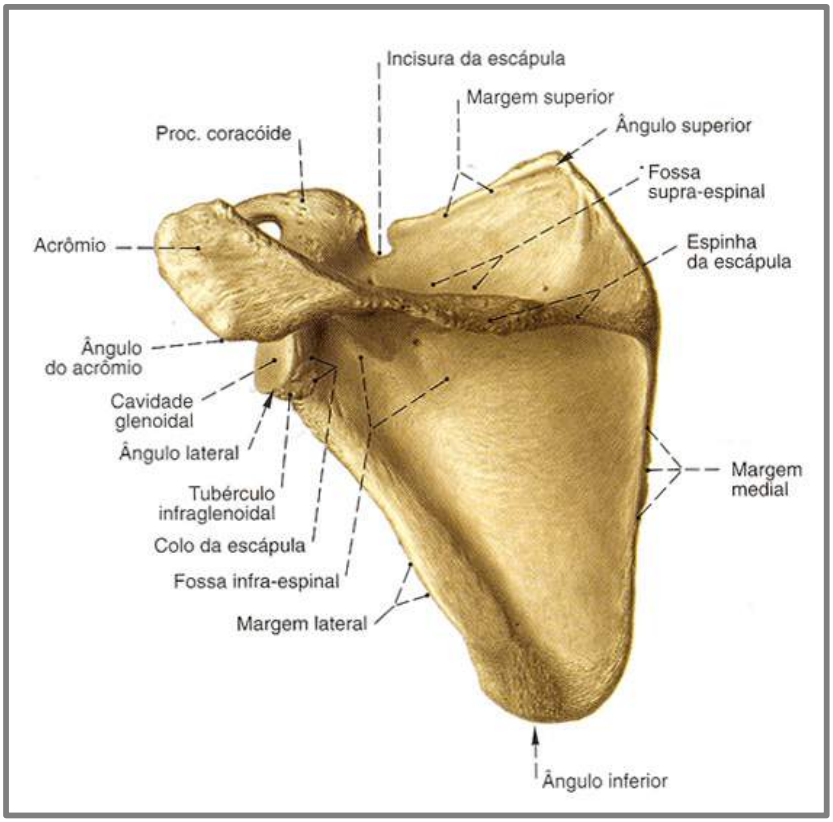

Figura 1 - Osteologia da escápula. Netter F. H. Atlas de Anatomia Humana – 6^{a} edição. Editora Elsevier. 2015 (Netter, 2015).

A escápula também é considerada o ponto de origem de todos os músculos intrínsecos e extrínsecos que dinamicamente estabilizam a AGU em quase todos os arcos de movimento e que são, no seu conjunto, responsáveis por até 90% do

movimento da AGU nos diferentes planos (Kibler & Sciascia, 2016). O manguito rotador atua comprimindo o centro da cabeça umeral com a glenoide diminuindo assim movimentos de translação (Lippitt et al, 1993).

Em adição, a escápula atua otimizando a posição da escápula e os movimentos necessários para limitar as cargas sobre os ligamentos e outras estruturas de contenção passivas da articulação. O aumento da protração da escápula cria sobrecarga excessiva sobre o ligamento glenoumeral inferior, aumentando o risco de instabilidade glenoumeral. Do mesmo modo, o aumento da inclinação anterior da escápula aumenta a compressão e as cargas de cisalhamento sobre a porção posterior e superior do lábio da glenoide.

Dessa forma, a escápula está envolvida na manutenção da estabilidade glenoumeral, proporcionando a eficiência máxima dinâmica, com a finalidade de manter a cinemática do conjunto “bola/soquete” (Kibler & Sciascia, 2016).

Discinesia da escápula é uma alteração da posição da escápula em repouso ou em movimento, levando à piora do ritmo escápulo-umeral. Apesar de discenesias não estarem associadas diretamente à instabilidade da AGU, essas modificações da posição ou dinâmica do movimento da escápula são frequentes em pacientes com instabilidade da AGU, ocorrendo em até 70 a 80% dos pacientes (Warner et al, 1992; Paletta et al, 1997). A presença da discinesia pode criar ou exacerbar os sintomas e disfunções da instabilidade e deve ser considerada no tratamento da instabilidade dessa articulação (Kibler & Sciascia, 2016).

O tipo de instabilidade pode ser um fator causal de discinesia, o que resulta em discinesia devido à dor ou alterações musculares (Kibler et al, 2007). Já em pacientes com instabilidade devido a microtraumas repetitivos ou lesão labral superior, o enfraquecimento e inibição do músculo trapézio inferior e serrátil anterior, mais a inflexibilidade do peitoral menor, são os principais responsáveis pela discinesia escapular (Burkhart et al, 2003). Na instabilidade multidirecional, a discinesia escapular e a ativação muscular alterada são ainda mais relevantes.

1.3.2.2 Adesão e coesão da AGU

Adesão-coesão é um mecanismo de estabilização pelo qual as superfícies articulares umedecidas com fluido articular se mantêm juntas por força de atração molecular do fluido por si mesmo e as superfícies articulares (Rockwood et al, 2009). O fluido articular tem a propriedade de alta resistência à tensão (dificulta a separação de superfícies recobertas por ele) e baixa resistência ao cisalhamento (facilita deslizamento entre superfícies). A superfície articular, como outras superfícies, tem a capacidade de manter fluidos aderidos. Quando duas superfícies que aderem fluidos estão em contato, a adesão do fluido à superfície e a coesão dos fluidos tendem a manter as duas superfícies juntas. Diversos estudos tem demonstrado que existe pressão negativa na AGU (Gibb et al, 1991). Embora a pressão negativa da AGU seja importante em todas as direções, a sua neutralização predispõe principalmente à luxação inferior do ombro (Yamamoto et al, 2006). No entanto, esse fator não parece importante na instabilidade anterior recorrente (Rockwood et al, 2009). A pressão negativa é máxima com os ombros em franca elevação. Quando o volume articular aumenta, a pressão intra-articular fica menos sensível a cargas externas.

Por outro lado, o mecanismo estabilizador da pressão negativa é reduzido por qualquer situação que diminua as propriedades do fluido articular acima citadas. Assim, inflamação de qualquer natureza ou diminuição da área de contato da AGU é capaz de reduzir o efeito da adesão-coesão.

1.3.2.3 Lábio da glenoide

O lábio da glenoide é uma estrutura fibrosa e fibrocartilaginosa que circunda e cobre a glenoide de forma circular, compreendendo as regiões superior e inferior, que são morfologicamente diferentes entre si (Itoigawa & Itoi, 2016) (Figura 2). Morfologicamente, pode ser dividido em duas regiões: a porção superior e a inferior. A porção superior tem aspecto meniscoide, é móvel e frouxamente ligada à glenoide. Já a região inferior é geralmente uma estrutura redonda, elevada, fibrosa e firmemente ligada à glenoide. A porção superior do lábio insere-se conjuntamente com o cabo longo do bíceps no tubérculo supraglenoidal.

O lábio é essencial na estabilidade da articulação glenoumeral, pois sua presença duplica a profundidade anteroposterior da cavidade glenoidea e aumenta

consideravelmente sua concavidade no plano superior-inferior (Itoigawa & Itoi, 2016). Em estudo em cadáveres, a ressecção do lábio reduziu em 20% a resistência à translação, quando a AGU era submetida à cargas compressivas (Soslowsky et al, 1992; Lippitt et al, 1993). O lábio também é importante para aumentar a superfície de contato com a cabeça umeral e, principalmente, atua como ponto de inserção dos ligamentos glenoumerais (Itoigawa & Itoi, 2016).

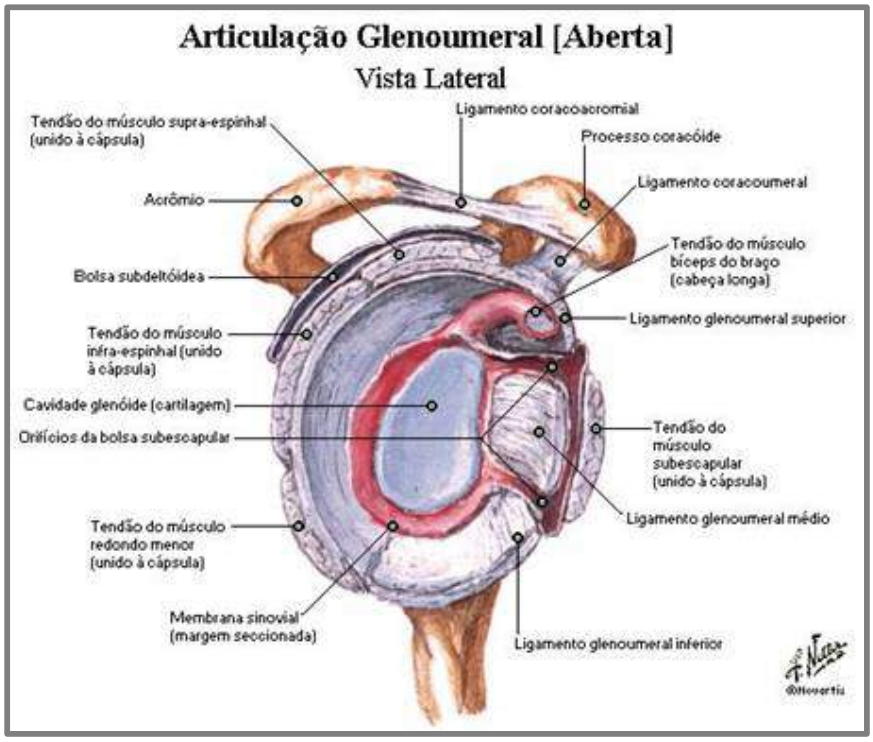

Figura 2 – Vista em perfil da articulação glenoumeral. Modificada de Netter F. H. Atlas de Anatomia Humana – 6ª edição. 2015 (Netter, 2015).

As alterações anatômicas decorrentes da luxação anterior traumática da AGU envolvem, regularmente, lesão por arranchamento do lábio anterior da glenoide associado à lesão plástica da cápsula anteroinferior da mesma articulação. Esses dois achados anatômicos, juntamente com a lesão de Hill-Sachs, representam as principais alterações morfológicas das luxações da AGU (Randelli et al, 2016).

1.3.2.4 Complexo cápsula, ligamentos e intervalo rotador

O complexo capsuloligamentar consiste das porções superior, média e inferior acrescidos do ligamento coracoumeral. A anatomia dos ligamentos glenoumerais é complexa e variável, sendo suas funções altamente dependentes da posição do úmero

em relação à glenoide (Burkart & Debski, 2002; Randelli et al, 2016). Esses ligamentos não atuam como ligamentos tradicionais que sofrem uma tensão única ao longo de seu trajeto, mas tornam-se tensos em variadas posições de abdução e rotação do úmero (Burkart & Debski, 2002). Nos extremos dos movimentos, os músculos tendem a estar em extensão máxima, posição em que suas capacidades de gerar força estão diminuídas. Nessas situações, cabe aos ligamentos glenoumerais proverem a compressão necessária à AGU (Rockwood et al, 2009). A região anteroinferior da cápsula glenoumeral é a porção mais frequentemente acometida em episódios de luxação traumática dessa articulação (Wang & Flatow, 2005). Após o primeiro episódio de luxação, é comum a presença de instabilidade crônica e recidivante. (Larrain et al, 2001; te Slaa et al, 2004). Pacientes com instabilidade da AGU apresentam deformidade plástica da cápsula que leva à frouxidão capsular (Hovelius et al, 1983; Wang & Flatow, 2005). Estudos prévios demonstraram que a deformação plástica da cápsula é necessária mesmo para ocorrência do primeiro episódio de luxação (Bigliani et al, 1992).

1.3.2.4.1 Ligamento glenoumeral superior

O ligamento glenoumeral superior (LGUS) é uma estrutura bem constante, presente em até 97% em estudos anatômicos (Rockwood et al, 2009). Seu tamanho é bem variado: desde uma fina camada de tecido capsular a até um espesso ligamento semelhante ao ligamento patelofemural do joelho. O LGUS consiste de fibras diretas e oblíquas, com diferentes origens e inserções. As fibras diretas originam-se do lábio da glenoide e as oblíquas no tubérculo supraglenoidal. As fibras diretas correm paralelamente com o tendão da cabeça longa do bíceps e as oblíquas correm sobre a porção intra-articular desse tendão. As fibras diretas inserem-se parcialmente na tuberosidade menor, formando a parte superior do ligamento umeral transverso. As fibras oblíquas inserem-se sob o ligamento coracoumeral, em direção ao ligamento umeral semicircular.

O LGUS contribui muito pouco para a estabilidade estática da AGU (Rockwood et al, 2009). No ombro abduzido, não afeta a translação anterior ou posterior da cabeça umeral. No entanto, LGUS é um estabilizador importante no sentido anterior e inferior, quando o ombro está aduzido e faz parte do mecanismo de estabilização da porção intra-articular do cabo longo do músculo bíceps braquial (Itoigawa & Itoi, 2016).

1.3.2.4.2 Ligamento glenoumeral médio

O ligamento glenoumeral médio (LGUM) apresenta grande variação em frequência e aspecto morfológico (Rockwood et al, 2009). Pode estar ausente em 12 a 27% das casuísticas estudas. Quando presente, origina-se do lábio, imediatamente abaixo ou junto do LGUS. LGUM insere-se no úmero, medialmente à tuberosidade menor, sob o tendão do músculo subescapular, ao qual se adere (Rockwood et al, 2009; Itoigawa & Itoi, 2016).

O LGUM deve contribuir para a estabilidade anterior, visto que se torna tenso em abdução de 45^{o} e em 10^{o} de extensão e rotação externa (Itoigawa & Itoi, 2016). A incorporação do LGUM no reparo de Bankart é polêmica e nenhuma pesquisa clínica demonstrou seu efeito até o momento (Rockwood et al, 2009; Itoigawa & Itoi, 2016).

1.3.2.4.3 Complexo ligamentar glenoumeral inferior

O complexo ligamentar glenoumeral inferior (LGUI) é o principal estabilizador estático quando o ombro está abduzido (Rockwood et al, 2009). Originalmente foi descrito como uma estrutura de forma triangular, com ápice no lábio e base se juntando à cápsula entre os músculos tríceps e subescapular.

Após a introdução da artroscopia, pôde ser mais bem descrito, definindo-se uma banda anterior, uma posterior e o recesso axilar entre as bandas (Rockwood et al, 2009). A banda anterior tem dois padrões distintos de ligação à glenoide: um padrão principal (padrão Tipo 1), que apresenta ligação ao lábio e algumas fibras se estendendo até o colo da glenoide, e o padrão Tipo 2, que apresenta ligação somente ao colo da glenoide (Itoigawa & Itoi, 2016). A porção anterior do LGUI limita a porção média da AGU a 90^{o} de abdução e em rotação externa, restringindo a translação anterior e inferior do úmero (Itoigawa & Itoi, 2016).

A banda posterior do LGUI é o estabilizador mais importante quando o ombro está em rotação interna com aplicação de carga em sentido posterior. A cápsula posterior que contem a banda posterior é relativamente fina, diferente da porção anterior, mais espessa. Da mesma forma, o desempenho biomecânico da cápsula póstero-inferior não é tão robusto quanto o da cápsula anterior. Em esportes de arremesso, a fadiga dos músculos posteriores pode levar ao aumento da tensão da cápsula posterior e da porção posterior do LGUI.

1.3.2.4.4 Ligamento coracoumeral

O ligamento coracoumeral (LCU) conecta o processo coracoide e o úmero. O LCU origina-se da base lateral e anterior do coracoide e estende-se como duas bandas sobre o topo do ombro convergindo com a cápsula e ligando-se às tuberosidades maior e menor (Rockwood et al, 2009). O LCU faz parte do arco coracoacromial que está em contato com a superfície superior do manguito rotador. Vários estudos tiveram como objetivo entender a relação da rotura do manguito rotador e a repercussão sobre o LCU ou vice-versa (McFarland et al, 2013; Familiari et al, 2015). Importa informar que a regeneração desse ligamento é lenta e suas funções ainda não totalmente esclarecidas (Rockwood et al, 2009).

A importância desse ligamento como estabilizador superior do ombro foi inicialmente descrita por Flatow et al (Flatow et al, 1996). Estudos posteriores também relataram aumento da translação da cabeça umeral após a liberação do LCU (Lee et al, 2001). No entanto, o papel do LGU como estabilizador ainda não é completamente compreendido. Enquanto alguns autores citam sua importância na rotação externa, contribuindo para impedir a subluxação inferior (Itoi et al, 1998), outros não encontram nenhum papel (Moorman et al, 2012).

1.3.2.4.5 Intervalo rotador

O intervalo rotador (IR) é um espaço triangular localizado na região anterossuperior da AGU. IR é limitado pela borda anterior do tendão do músculo supraespinhal superiormente, pelo tendão do músculo subescapular inferiormente e a base do coracoide, medialmente. O espaço contém o LCU, LGUS, o cabo longo do bíceps e parte da cápsula articular (Harryman et al, 1992; Itoi et al, 1998).

A estrutura lateral do IR inclui a primeira camada correspondente às fibras do LCU, seguida das inserções dos músculos subescapular e supraespinhal. A segunda camada é composta de fibras ligadas à musculatura do manguito rotador e do LCU. A terceira camada inclui fibras profundas do LCU inseridas na tuberosidade maior. Mais profundamente há a cápsula articular e LGUS (Harryman et al, 1992).

A estrutura medial do IR tem duas camadas, uma superficial, que corresponde ao LCU e a camada profunda incluindo o LGUS e a cápsula articular (Itoigawa & Itoi, 2016).

Diversos estudos têm demonstrado que o IR tem um papel significativo na estabilidade da AGU, embora sem conhecimento preciso dos mecanismos envolvidos. Secção do IR, experimentalmente, leva a aumento real da translação anterior, posterior e inferior da cabeça umeral. Por outro lado, um estudo em cadáver demonstrou que o fechamento do IR não contribuiu significativamente para a redução do arco de movimento, mas tem implicações na translação glenoumeral (Harryman et al, 1992; Itoigawa & Itoi, 2016).

Do ponto de vista qualitativo, a cápsula da AGU pouco difere da localizada em outras articulações e consiste de colágeno do tipo I, III e V. A cápsula é fina e, embora de modo variável, apresenta redundância (Rockwood et al, 2009). A cápsula articular é conhecida como um fator associado a maior ou menor frouxidão e à predisposição à instabilidade. As cadeias colágenas se organizam por meio de ligações cruzadas (*crosslink*) e além disso, outras proteínas da matriz extracelular também tem grande importância para a estrutura e homeostase desse tecido.

1.4 Instabilidade da articulação glenoumeral

A instabilidade da AGU é definida como uma sensação desagradável, referida pelo paciente quando seu ombro está na iminência de luxar (Rockwood et al, 2009). O termo luxação significa a perda completa do contato entre duas superfícies articulares e pode ser ocasionado por evento traumático ou voluntário (Rockwood et al, 2009). Após o primeiro episódio de luxação do ombro, o indivíduo pode evoluir para a estabilidade ou instabilidade da articulação. Aqueles que evoluem para manutenção dos sintomas de instabilidade estão sujeitos a sofrerem luxações repetidas, ou recidivantes (Rockwood et al, 2009).

Hipócrates, entre 3000-2500 anos A.C. descreveu a redução de um ombro luxado usando o calcanhar na axila e aplicando tração no braço afetado. Também descreveu o uso de um ferro *red hot* inserido na axila para causar aderência na porção inferior da articulação para tratamento da instabilidade recorrente.

A elevada frequência da instabilidade da AGU na população é um grande estímulo para seu estudo. As luxações anteriores ocorrem em cerca de 2% da população mundial, sendo que 80% desses casos ocorrem em pacientes jovens (Owens et al, 2007). É muito

comum em atletas, principalmente em jovens com menos de 20 anos envolvidos em esportes competitivos de contato (Buss et al, 2004).

A instabilidade recorrente após o primeiro episódio de luxação é frequente, com relatos, em populações de risco, de até 100% (Larrain et al, 2001; te Slaa et al, 2004). A incidência de luxações recorrentes é altamente dependente da idade: ocorre em cerca de 66 a 100% em menores de 20 anos, 13 a 63% entre 20-40 anos e 0 a 16% em maiores de 40 anos (Rowe & Zarins, 1981; Lebus et al, 2015; Olds et al, 2015). A repercussão dessa doença em populações de atletas é ainda exacerbada (Veltri, 2010; Murray et al, 2013).

1.5 Bases genéticas das doenças de herança complexa

A hereditariedade contribui para muitas doenças humanas comuns na população, como, por exemplo, defeitos congênitos, infarto do miocárdio, câncer, doenças mentais, diabetes e grande parte das doenças ortopédicas. Embora as doenças ortopédicas até possam ser causadas por uma mutação em um único gene em algumas famílias, em geral, elas não são monogênicas. A maioria dessas afecções é o resultado de interações complexas entre vários fatores de predisposição, como os genótipos de diferentes *loci* e uma variedade de fatores ambientais que ativam, aceleram ou exacerbam o processo da doença. Assim, essas doenças, como a IATO, não podem ser enquadradas em padrões de herança mendelianos, mas sim em padrões complexos ou multifatoriais (interação gene-ambiente). Uma caraterística das doenças complexas, incluindo a IATO, é que elas podem apresentar agregação familiar, pois é mais provável que os parentes de uma pessoa afetada compartilhem com ela mais alelos de predisposição à doença do que pessoas não-aparentadas. Compreender os mecanismos pelos quais os alelos de mais de um *locus* conferem suscetibilidade/proteção à doença ou como esses alelos contribuem para a variação fenotípica é de suma importância (Nussbaum et al, 2008).

Nosso grupo de pesquisa busca estudar os mecanismos genéticos e transcricionais envolvidos no desenvolvimento de diversas lesões ortopédicas, incluindo a IATO.

1.5.1 Bases genéticas e aspectos translacionais da articulação glenoumeral

Para um maior entendimento, dissertaremos sobre os genes do presente estudo, agrupando-os em três grupos: 1) genes codificadores de colágeno; 2) genes relacionados com modulação de síntese de fibras de colágenos e a estabilização das fibrilas de colágeno (genes de *crosslink*) e 3) genes de glicoproteínas da matriz extracelular.

1.5.1.1 Genes codificadores de colágeno

1.5.1.1.1 Genes envolvidos na fibra de colágeno tipo I: *COL1A1* e *COL1A2*

O colágeno tipo I, dentre todos os tipos de colágeno, é o mais abundante e encontrado na maioria dos tecidos de conexão sendo, preferencialmente, sintetizado em ossos (forma 90% da matriz extracelular óssea) (Prokop et al, 1998), derme e tendões por dois tipos de células: os osteoblastos e os fibroblastos (Karsenty & Park, 1995).

O colágeno tipo I é uma molécula heterotrímera composta de duas cadeias alfa1 e uma cadeia alfa2 que se entrelaçam formando uma rígida tripla hélice (Figura 3) (Karsenty & Park, 1995; Bennett & Plum, 1997; Prokop et al, 1998; Cotran et al, 2000). O gene *COL1A1,* localizado na região genômica 17q21.33, codifica as cadeias pro-alfa1 do colágeno tipo I enquanto o gene *COL1A2,* localizado na região genômica 7q22.1, sintetiza a cadeia alfa2.

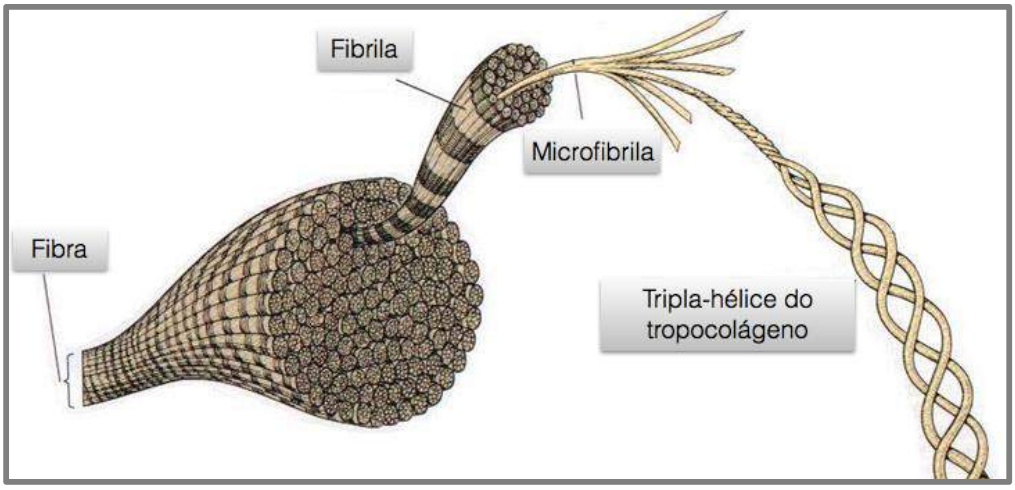

Figura 3 – Estrutura do colágeno: da tripla-hélice à fibra (modificado de Campbell NA. Biology 1995. Éditions Du Renouveau Pédagogique Inc. St Laurent) (Campbell, 1995) .

Fibras de colágeno que formam o colágeno tipo I são sintetizadas em precursores maiores, conhecidos como pró-colágenos, que contêm pró-peptídeos globulares N-terminal e C-terminal. Duas cadeias pró-1 e uma pró-2 associam-se primeiro pelas interações hidrofóbicas e eletrostáticas entre os pró-peptídeos C (de Wet et al, 1987; Kuivaniemi et al, 1988).

Essa duas cadeias polipeptídicas são sintetizadas em uma proporção 2:1, e essa mesma proporção é observada na síntese de RNAs mensageiros (mRNAs) correspondentes (Karsenty & Park, 1995). A expressão do colágeno tipo I está aumentada em várias formas de fibroses, tais como pulmonar, hepática e da medula óssea (Karsenty & Park, 1995). Mutações em genes de colágeno I têm sido descritas em diferentes doenças genéticas.

Em uma extensa revisão, Marini et al. (2007) identificaram 832 mutações independentes nos genes do colágeno 1 (493 no *COL1A1*). Um terço das mutações que resultam em substituições de glicina no *COL1A1* foram letais, enquanto que substituições nos 200 primeiros resíduos foram não letais e não relacionados com a aumento ou a diminuição da expressão (Marini et al, 2007). Mutações de *COL1A1* estão associadas com doenças tais como a osteogênese imperfeita (OI) tipo I-IV, a síndrome Ehlers-Danlos (EDS), a doença de Caffey e a osteoporose idiopática (Starman et al, 1989; D'Alessio et al, 1991; Grant et al, 1996; Kamoun-Goldrat et al, 2008). A maioria das mutações no *COL1A1* altera a estrutura primária do colágeno, já algumas outras, resultam em um alelo nulo de *COL1A1* e, embora só metade da quantidade normal do pró-colágeno tipo I torna-se sintetizada, a sua estrutura é normal. Quase todas as moléculas que contêm cadeias com mutações em genes do colágeno tipo I são menos estáveis do que as cadeias normais (Baker et al, 1989).

Marini et al. (2007) também descreveram 339 mutações independentes nos em *COL1A2*. Mutações no *COL1A2* foram predominantemente não letais (80%). Assim como as mutações no gene *COL1A1*, as mutações no *COL1A2* estão associadas à OI, EDS e também com síndrome de Marfan atípica. Sintomas associados com mutações nesse gene tendem a ser mais brandos do que os sintomas relacionados ao gene *COL1A1*, refletindo um papel diferente das cadeias alfa2 na integridade da matriz (Kojima et al, 1988; Schwarze et al, 2004).

Variantes genéticas comuns (polimorfismos de DNA) no gene *COL1A1* parecem ser potenciais fatores genéticos de risco para diferentes doenças, incluindo lesões ortopédicas, tais como as roturas do ligamento cruzado anterior (LCA) e luxações do ombro (Khoschnau et al, 2008). Collins et al., juntando os resultados de indivíduos caucasoides da África do Sul e Suíça, investigaram se um polimorfismo no sítio de ligação Sp1 de *COL1A1* estava associado ao risco de lesão do ligamento cruzado, instabilidade de ombro e roturas do tendão de Aquiles. Os autores descreveram que o genótipo TT do polimorfismo funcional do primeiro íntron do gene *COL1A1*, era um fator protetor para lesão do tendão de Aquiles. O mesmo resultado foi obtido quando todas as lesões foram combinadas e comparadas a indivíduos controles.

Posthumus et al. Também demonstram que um polimorfismo de *COL1A1* (rs180012) era fator protetor para lesão do LCA em uma população caucasoide da África do Sul (Posthumus et al, 2009a). Por outro lado, Posthumus et al. não identificaram associação entre esse polimorfismo e rotura do tendão de Aquiles (Posthumus et al, 2009b). Em uma população da Suécia, rs1800012 foi associado ao risco de lesão do LCA e instabilidade de ombro (Khoschnau et al, 2008). Esses achados apontam um papel de genes de colágenos em lesões de LCA e instabilidade de ombro, além de outras afecções ortopédicas associadas à hipermobilidade das articulações.

1.5.1.1.2 Gene *COL3A1*

A cadeia alfa do colágeno tipo III é codificada pelo gene *COL3A1*, localizado em 2q31. Esse tipo de colágeno é encontrado em tecidos flexíveis de conexão e, frequentemente, está em associação com o colágeno tipo I. Esse gene é expresso em estágios bem precoces da embriogênese e estende-se por toda ela. Mutações nesse gene estão associadas a EDS e a aneurismas arteriais e aórticos (Liu et al, 1997). Como já mencionado, nos ligamentos e tendões, o colágeno é a proteína mais importante e abundante e nessas estruturas, em recém-nascidos, o colágeno tipo III (solúvel) é sintetizado e suas fibras formadas são maleáveis e elásticas. Com o passar das décadas, as células produtoras de colágeno sintetizam menos tipo III e, progressivamente, mais tipo I, que é insolúvel e por isso, mais estável (Liu et al, 1997).

O colágeno tipo I apresenta grupos sulfato que tem a tendência de fazer ligações do tipo *crosslink* (ligação cruzada) e pontes com outros filamentos colágenos, tornando o tecido menos elástico e mais estável. A alteração na razão da produção de colágeno

tipo I e colágeno tipo III está altamente relacionada à idade cronológica (Hayes et al, 2002). Sendo assim, a maior concentração de colágeno tipo III em tendões e ligamentos de jovens, tornam essa população mais predisposta à instabilidade recorrente do que populações mais velhas (Hayes et al, 2002). Sendo assim, maior expressão de colágeno III em ligamentos, ou mesmo em outros tecidos articulares,de indivíduos adultos pode estar relacionada à hipermobilidade ou instabilidade de articulações e, dessa forma, ao risco de lesões.

Imazato (1992) e Hirakawa (1991), em estudos independentes, demonstraram que em pacientes com instabilidade multidirecional do ombro, as fibras colágenas da cápsula, músculos e pele são relativamente imaturas, mais solúveis e com menos *crosslink* que controles (Hirakawa, 1991; Imazato, 1992).

1.5.1.1.3 Gene *COL5A1*

O gene *COL5A1* está localizado em 9q34.2 e sintetiza uma cadeia alfa para um dos colágenos fibrilares de baixa abundância, o colágeno V. A maior isoforma de colágeno V é um heterotrímero constituído por duas cadeias de α1 (produto do gene *COL5A1*) e uma de α2 (produto do gene *COL5A2*), porém algumas isoformas podem conter uma cadeia α3 (produto do gene *COL5A3*).

O colágeno tipo V, que constitui aproximadamente 10% do conteúdo de colágeno nos ligamentos (Niyibizi et al, 2000), se intercala nas fibrilas do núcleo do colágeno tipo I. Esse colágeno parece estar envolvido na organização e na regulação das fibras de colágeno tipo I (Niyibizi et al, 2000). O colágeno tipo V também está fortemente relacionado ao colágeno tipo XI e é possível que as cadeias de colágeno dos tipos V e XI constituam um único tipo de colágeno com combinações de cadeia tecido-específicas.

O colágeno tipo V é encontrado em tecidos que contém colágeno tipo I e parece regular a organização das fibras heterotípicas compostas de ambos colágenos, tipo I e tipo V (Fichard et al, 1995). Mutações em genes codificantes de colágeno tipo V também estão associadas a EDS,tipos I e II e VII (Nicholls et al, 1996). Um recente estudo realizado com sequenciamento de nova geração identificou diversas variantes nunca antes descritas no gene *COL5A1* em 177 pacientes portadores de EDS não aparentados (Weerakkody et al, 2016).

Lesões articulares são condições complexas e por isso, é improvável que uma única variante genética esteja associada ao seu risco (September et al, 2007). O gene *COL5A1* já foi associado como um fator de risco genético para as roturas do LCA (September et al, 2007) Mais estudos ainda são necessários para entender o envolvimento de *COL5A1* em lesões ortopédicas, incluindo na IATO.

1.5.1.2 Genes relacionados com a estabilização das fibrilas de colágeno (genes de *crosslink*)

As fibrilas de colágenos são estabilizadas por ligações covalentes entre moléculas, chamadas *crosslinks* (Figura 4). A formação de *crosslinks* é facilitada pela organização precisa das moléculas de colágeno dentro das fibrilas, governadas por interações hidrofóbicas e eletroestáticas. Diferentes tipos dessas ligações são descritas na literatura, e as suas estruturas, números e localizações são, em geral, tecido específicas (Ha-Vinh et al, 2004). Lisina e hidroxilisina são os principais resíduos de aminoácido envolvido em *crosslinks* de colágenos. Dessa forma, a força tênsil da matriz de colágeno é controlada não somente pelo tamanho das fibrilas, mas também pela formação dessas ligações covalentes envolvendo resíduos de lisina e hidroxilisina dentro e entre moléculas de colágeno (Robins, 2007).

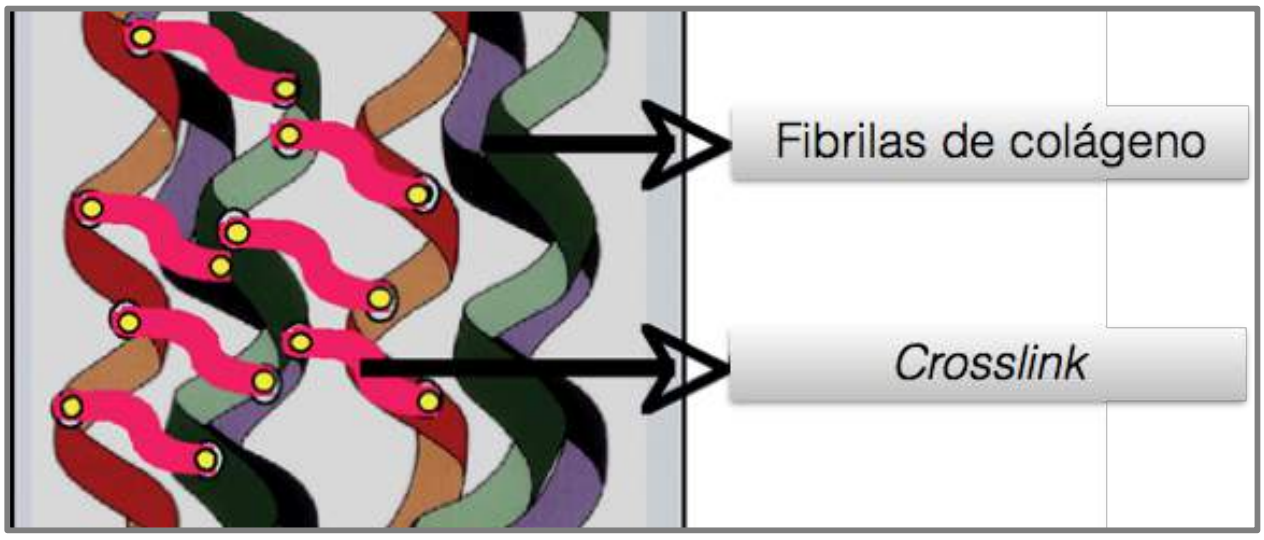

Figura 4 - Esquema de representação do processo de *crosslink* entre as fibrilas de colágeno. Modificado de Nataraj et al., 2007 (Nataraj et al, 2007).

Como descrito anteriormente, dois estudos independentes demonstraram que a cápsula glenoumeral de pacientes com instabilidade multidirecional apresenta fibras

colágenas mais solúveis e com menos *crosslink* em comparação com indivíduos controles (Hirakawa, 1991; Imazato, 1992).

1.5.1.2.1 Gene *LOX*

A lisil oxidase (LOX) é uma enzima envolvida no processo de estabilização de fibrilas de colágeno e outras proteínas da matriz extracelular (MEC). Essa enzima é codificada pelo gene *LOX*, localizado em 5q23.1.

LOX catalisa a formação de 20-lisil-aldeído (alisina) e hidroxilisil-aldeído (hidroxialisina) e é responsável pelo primeiro passo da formação de *crosslinks* de colágeno. Todas as reações seguintes para a formação de *crosslinks* envolvendo moléculas de colágeno ocorrem espontaneamente em virtude do alinhamento dessas moléculas.

LOX também catalisa a desaminação oxidativa de resíduos peptidil-lisina de precursores de elastina (Smith-Mungo & Kagan, 1998; Csiszar, 2001). Fibronectina (FN) também parece ser um possível alvo de LOX (Fogelgren et al, 2005). A ligação cruzada entre essas proteínas de MEC por LOX é essencial para a formação de colágeno insolúvel e fibras elásticas e para o desenvolvimento normal de mamíferos (Smith-Mungo & Kagan, 1998; Csiszar, 2001).

A atividade de LOX é essencial para a manutenção das características elásticas e a resistência tênsil de tecidos conectivos, pulmonar e do sistema cardiovascular, entre outros (Ma et al, 2011). Hornstra et al. (2003) demonstraram que LOX é importante para o desenvolvimento da resistência à tração em tecidos conjuntivos (Hornstra et al, 2003). Cox et al. (2015) identificaram LOX como um novo regulador de NFATc1-osteoclastogênese dirigido que é capaz de perturbar a homeostasia óssea normal (Cox et al, 2015). Dessa forma, LOX parece atuar na regulação da homeostase óssea e pode, também, ter um papel em outros tecidos articulares.

A expressão e a atividade de LOX são alteradas em muitas doenças humanas, incluindo câncer, doenças cardiovasculares e fibrose (Fogelgren et al, 2005).

1.5.1.2.2 Genes que codificam lisil hidroxilases: *PLOD1 e PLOD2*

A hidroxilisina livre não pode ser incorporada às moléculas de colágeno. Dessa forma, as enzimas lisil hidroxilase (LH) são responsáveis pela hidroxilação de resíduos de lisina dentro do heterotrímero de colágeno e em telopeptídeos amino e carboxil-terminais (Ha-Vinh et al, 2004). Em humanos, há três genes que codificam isoformas de LH: *PLOD1* (mapeado em 1p36.22), *PLOD2* (localizado em 3q23-q24) e *PLOD3* (localizado em 7q22.1), que codificam as isoformas LH1, LH2 e LH3, respectivamente. Uma variante de RNAm transcrito por *PLOD2* é encontrada no rim fetal e pâncreas (Valtavaara et al, 1997), uma segunda variante, isolado a partir de fibroblastos de pele humana (Yeowell & Walker, 1999). Estas duas formas de RNAm são referidas como LH2a e LH2b, respectivamente.

Resíduos hidroxilisina em telopeptídeos são convertidos em hidroxialisinas, que, em seguida, reagem com resíduos de lisina e hidroxilisina da tripla hélice para formar *crosslinks* di-, tri-, ou tetra-funcionais. Esses *crosslinks*, derivados tanto de um resíduo de hidroxilisina e outro de lisina ou de dois resíduos de hidroxilisina, são encontrados em uma variedade de tecidos conjuntivos (Eyre et al, 1984). A quantidade de hidroxilisina, assim como a razão de hidroxilisina paralisina, variam entre os diferentes tipos de colágeno.

A enzima LH1 catalisa a formação de hidroxilisina em colágenos e outras proteínas *colagen-like*. Os resíduos formados têm duas importantes funções: na primeira, seus grupos de hidroxi servem como sítios de ligação para unidades de carboidratos ou galactose monossacarídea ou glicogalactose dissacarídea; na segunda, eles são essenciais para a estabilidade de *crosslink* de colágenos (Hautala et al, 1992).

Pacientes com EDS tipo 6 apresentam mutação em *PLOD1* que resulta na redução da atividade de LH1, ressaltando a importância de LH1 na estrutura de tecidos conjuntivos (Giunta et al, 2005). Recentemente, uma mutação nova no gene *PLOD1* foi descrita em uma criança portadora de Ehlers-Danlos (Tosun et al, 2014)

A alteração da expressão de LH2 parece estar envolvida em algumas doenças de colágeno, como na síndrome de Bruck e em processos de fibrose. Adicionalmente, em duas famílias com síndrome de Bruck, van der Slot et al. (2003) identificaram duas diferentes mutações *missense* em homozigose no gene *PLOD2* (van der Slot et al, 2003).

Puig-Hervas et al. (2012) estudaram mutações em 6 famílias egípcias não relacionadas com síndrome de Bruck e identificou alterações em homozigose no gene *PLOD2* em quatro famílias. Puig-Hervas et al. (2012) também identificaram uma mutação em homozigose no *PLOD2* (1358 + 5G-A) em um paciente egípcio com o que os autores chamaram de OI autossômica recessiva e mutações em heterozigose em dois irmãos espanhóis, sendo que um teve o diagnóstico de OI autossômica recessiva e outro teve o diagnóstico de síndrome de Bruck leve (Puig-Hervas et al, 2012). Puig-Hervas et al. (2012) sugeriu que *PLOD2* causa a síndrome de Bruck, bem como OI autossômicas recessivas de gravidade variável.

Apesar de não terem sido descritas mutações de *PLOD2* em pacientes com EDS, uma reduzida expressão do seu mRNA foi observada em fibroblastos da pele de pacientes com essa síndrome que apresentavam níveis normais de LH1 e LH3 (Walker et al, 2004).

1.5.1.3 Genes relacionados com a modulação da síntese de fibras de colágeno: *TGFB1 e TGFBR1*

O gene *TGFB1*, localizado em 19q13.2, codifica um peptídeo multifuncional (TGFβ) que controla a proliferação, a diferenciação e outras funções em muitos tipos de células. TGFβ também atua como um fator de crescimento autócrino negativo. A desregulação da ativação de sinalização de TGFβ pode resultar em apoptose.

Muitas células sintetizam TGFβ e quase todos eles possuem receptores específicos para esse peptídeo (Ebner et al, 1993). Vários tipos de ligantes de proteínas TGFβ foram detectados na superfície da célula. Os receptores Tipo I e Tipo II são definidos com base na mobilidade dos seus produtos de ligação cruzada em géis desnaturantes. Estes receptores medeiam a maioria das atividades de TGFβ. O receptor de tipo II (codificado pelo gene *TGFBR2*) funciona como um tirosina-serina/cinase transmembranar e é necessário para a atividade antiproliferativa de TGFβ, enquanto que o receptor de tipo I medeia a indução de vários genes envolvidos em interações célula-matriz (Ebner et al, 1993). O gene *TGFBR1*, mapeado em 9q22.33, codifica um receptor de serina/treonina cinase para TGFβ1.

A resposta inflamatória é normalmente a resposta mais precoce na reparação tecidual, seguida pela deposição de nova matriz de tecido conectivo (Larrain et al, 2001).

O aumento na transformação do fator de crescimento TGFβ1 acompanha a fase de inflamação aguda e parece agir como sinal de modulação da produção de macromoléculas de matriz por células fibrogênicas no local de lesão tecidual (Sakai et al, 2002). TGFβ1 regula importantes enzimas modificadoras do colágeno como a LOX e a lisil hidroxilase 1 e 2 (codificadas pelos genes *PLOD1* e *PLOD2* respectivamente) (Yoshida & Fujii, 1999; Knippenberg et al, 2009; Remst et al, 2014; Witsch et al, 2014).

Algumas manifestações da síndrome de Marfan refletem a sinalização excessiva pela família TGFβ de citocinas. Habashi et al. (2006) mostraram que o aneurisma aórtico num modelo de rato da síndrome de Marfan está associada com o aumento da sinalização de TGFβ (Habashi et al, 2006). Na doença de Dupuytren, uma doença caracterizada por um processo de fibrose na região volar das mãos, já foi descrito aumento da expressão de *TGFB1* e de seu receptor *TGFBR1*, além da associação desse gene com fibrose e acumulação de matriz densa de colágeno do tipo I e III. TGFβR1 também foi descrito como elemento fundamental na regulação da cicatrização tecidual (Liu et al, 2011). Dessa forma, hipotetizamos que a via de sinalização de TGFβ1 pode ter um importante papel em lesões ortopédicas.

1.5.1.4 Genes codificadores de glicoproteínas da matriz extracelular

Outros componentes não colagenosos de MEC de ligamentos/tendões, cápsula ou retinácula são pouco estudados (Riley, 2010). Em ligamentos/tendões e meniscos, as principais proteínas não colagenosas são as glicoproteínas fibronectina (FN), tenascina (TN) e proteínas oligoméricas da matriz da cartilagem (COMP) (Riley, 2010).

1.5.1.4.1 Gene *FN1*

O gene *FN1,* localizado na região 2q35, codifica a fibronectina-1, proteína pertencente a uma família de glicoproteínas de elevado peso molecular que encontram-se presente na superfície das células, em fluidos extracelulares, os tecidos conjuntivos e de membranas basais. Fibronectinas interagem com outras proteínas de MEC e ligantes celulares, tais como o colágeno, a fibrina, e integrinas. Fibronectinas estão envolvidos em processos adesivos e migratórios de células.

Uma das principais funções dos fibronectinas é na adesão de células aos materiais extracelulares, tais como substratos sólidos e matrizes. Bing et al. (1982) mostraram que a fibronectina se liga a C1q, da mesma maneira que se liga colagéno (Bing et al, 1982).

Uma vez que fibronectina estimula a endocitose e promove a eliminação de material em partículas a partir da circulação, Bing et al. (1982) sugeriram que as funções de fibronectina na depuração de material C1q-revestidos, tais como complexos imunes ou detritos celulares.

A fibronectina é a uma glicoproteína que participa ativamente da adesão celular, desenvolvimento tecidual e cicatrização tecidual (Vakonakis & Campbell, 2007). Essa proteína atua no processo de reparo dos tendões por promover migração de fibroblasto e adesão de fibroblastos às fibrilas. Tillander et al. (2002) observaram maior expressão de fibronectina em tendões do manguito rotador com rotura em comparação com amostras de controles saudáveis por análise de imunofluorescência. Depósito de FN também foi detectado em lesões do tendão calcâneo (Tillander et al, 2002; Vakonakis & Campbell, 2007). Dessa forma, hipotetizamos que fibronectina também pode ter um papel importante em lesões da cápsula glenoumeral.

1.5.1.4.2 Tenascinas: *TNC* e *TNXB*

Tenascina são proteínas da matriz extracelular com uma distribuição de tecido espacialmente e temporalmente restrita. No embrião, está presente no mesênquima em torno do epitélio em desenvolvimento, em tendões, e no desenvolvimento de cartilagem e osso. No adulto, ele continua presente nos tendões e junções miotendíneas no pericôndrio e periósteo, bem como no músculo liso (Pearson et al, 1988). As tenascinas estão envolvidas nos processos de morfogênese, migração e crescimento de diversos órgãos e tecidos (Tochigi et al, 2007).

As tenascinas formam uma família de grandes proteínas multiméricas de matriz extracelular. Foram identificados três membros dessa família em humanos: tenascina R (TNR), tenascina C (TNC) e tenascina X (TNXA e TNXB). O gene *TNXA* corresponde a um segmento duplicado de *TNXB* indo do íntron 32 ao éxon 45. A expressão da proteína TNR é restrita ao cérebro, enquanto TNC e TNX são expressas em diferentes órgãos (Zweers et al, 2004). Dessa forma, no presente estudo, somente TNC e TNX serão abordadas.

O gene *TNC*, que codifica TNC, está mapeado em 9q33.1. TNC é expresso em tendões, ligamentos e meniscos, entre outros tecidos (Riley, 2010). Em tendões, TNC, assim como fibronectina, atua no processo de reparo por promover migração de

fibroblasto e adesão de fibroblastos às fibrilas. Riley et al. descreveram um aumento da expressão de TNC em amostras com rotura do tendão do manguito rotador (N=10) comparadas a amostras de tendão sadio (N=13 de 9 cadáveres) (Riley et al, 1996). Em lesões do tendão calcâneo, variantes do gene *TNC* foram associadas ao aumento do risco de lesão e podem, dessa forma, contribuir para o remodelamento de matriz alterado em tendinopatias (Mokone et al, 2005). Midwood et al. (2009) descobriram que ratos sem *Tnc* não mostraram sinovite, infiltrado celular ou perda peptidoglicano em cartilagem em resposta ao zimosan e foram protegidos contra a destruição articular induzida por albumina do soro bovino metilada (Midwood et al, 2009). Assim, TNC pode ter um importante papel em lesões ortopédicas.

O gene *TNXB*, localizado em 6p21.33, codifica a TNXB, uma glicoproteína de MEC localizada, predominantemente, na lâmina reticular exterior da membrana basal (Penisson-Besnier et al, 2013). Menos se conhece sobre TNX em relação à TNC. Em humanos, TNX é expresso abundantemente em todos os tecidos conectivos, incluindo tendões e ligamentos, e um fragmento de140 kDa está presente no soro (Zweers et al, 2005).

TNX parece ser importante para a deposição apropriada das fibras de colágeno na derme. Mutações de *TNX*, resultando em deficiência dessa proteína, causam uma forma autossômica recessiva de EDS, que é uma doença do tecido conjuntivo caracterizada por hipermobilidade das articulações, hiperfrouxidão da pele e fragilidade de tecidos A deficiência de *TNX* está associada a luxações ou subluxações articulares em indivíduos com EDS (Zweers et al, 2004). TNX também é candidato à participar da síndrome da hipermobilidade articular benigna, na qual foi previamente descrita uma redução dos níveis séricos de TNX (Zweers et al, 2005). Estudos com fibroblastos de camundongos nulos para TNX sugerem que essa proteína tem um papel fundamental na regulação da deposição de fibrilas de colágenos, independentemente da síntese de colágenos e fibrilinogênese (Mao et al, 2002). TNXB foi descrito como candidato a regulação de síntese e deposição de colágeno (Zweers et al, 2004).

Embora tendões e ligamentos possuam poucas fibras elásticas, é possível que TNX também contribua para a manutenção da estabilidade de articulações por meio da regulação da estrutura dessas fibras em uma maneira dose-dependente (Zweers et al, 2004).

1.5.1.4.3 Gene *COMP*

O gene *COMP*, localizado em 19p13.11, codifica uma proteína proteína oligomérica da MEC (COMP, do inglês *cartilage oligomeric matrix protein*) que catalisa a montagem de colágenos e promove a formação de fibrilas bem definidas (Halasz et al, 2007). No entanto, a função do gene *COMP* ainda não é totalmente conhecida. A proteína COMP apresenta um sítio de ligação para as moléculas de colágenos I, II e IX (Mabuchi et al, 2003), o que sugere um papel estrutural e interativo. Essa proteína, membro da família de genes trombospondina é expressa em níveis elevados na matriz de condrócitos (Newton et al, 1994).

O papel estrutural de COMP foi demonstrado pela descoberta de mutações nesse gene na pseudo-acondroplasia, uma doença genética rara caracterizada por baixa estatura, frouxidão ligamentar e osteoartrose de acometimento precoce. Mutações em *COMP* também estão associadas à displasia epifisária múltipla (Hecht et al, 1995).

Em equinos, foi demonstrado que a expressão de COMP reduz com a maturidade do esqueleto. No entanto, um aumento da COMP é observado durante o processo de cura após uma lesão tendínea (Smith et al, 1997). Tem sido proposto que a detecção de fragmentos de COMP liberados no líquido sinovial e na corrente sanguínea pode ser um marcador de lesão do tendão, assumindo que esses fragmentos são derivados dos tendões e podem ser diferenciados dos derivados de cartilagem e outros tecidos (Smith et al, 1999). Assim, hipotetizamos que COMP também pode ter um papel na integridade de outros tecidos articulares, como a cápsula glenoumeral.

ARTIGOS CIENTÍFICOS

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Como mencionado anteriormente, esta tese originou quatro artigos científicos já publicados, sendo que, no primeiro (Belangero et al, 2014a), estudamos a expressão de genes do colágeno em diferentes regiões da cápsula articular em uma coorte de indivíduos com IATO. Nesse estudo, buscamos avaliar, pela primeira vez na literatura, a expressão gênica na cápsula desses pacientes. Avaliamos, inicialmente, a expressão de genes codificadores de colágeno em amostras da cápsula glenoumeral com alteração macroscópica (região AI) e sem alteração macroscópica (região AS). A apresentação desses dados de expressão de genes codificadores de colágeno no congresso do *International Society of Arthroscopy, Knee Surgery and Orthopaedic Sports Medicine* (ISAKOS) em Toronto em 2013 recebeu o prêmio de melhor trabalho (Anexo 3).

Já nos estudos seguintes (Belangero et al, 2014b; Belangero et al, 2016a; Belangero et al, 2016b), o número de indivíduos com IATO foi aumentado e incluímos um grupo controle externo (indivíduos sem IATO e sem alteração da cápsula glenoumeral). Com base nesses estudos, podemos observar que a cápsula de pacientes com IATO estava alterada como um todo, pois detectamos alterações na expressão gênica mesmo em regiões da cápsula sem alterações macroscópicas (AS e P).

No segundo estudo (Belangero et al, 2014b), continuamos estudando a expressão de genes de colágeno. Os resultados desse estudo nos incentivaram a realizar um terceiro estudo em que buscamos avaliar a expressão de genes que modulam a síntese de fibras de colágenos ou que estão envolvidos no *crosslink* de colágenos (Belangero et al, 2016a). Em seguida, realizamos o quarto estudo incluído na presente tese de doutorado (Belangero et al, 2016b), no qual avaliamos o papel de genes codificantes de outras proteínas não colagenosas da matriz extracelular na mesma população de pacientes e controles.

Esses estudos fazem parte de um projeto maior (FAPESP 2012/07721-2), no qual ainda será avaliada a expressão do produto proteico dos genes citados anteriormente. Esses projetos foram aprovados pelo Comitê de Ética em Pesquisa (Anexos 1 e 2). Adicionalmente, será avaliada a expressão de micro-RNA envolvidos na regulação da expressão desses genes. Nosso grupo busca ainda identificar variantes genéticas associadas ao risco de desenvolvimento de IATO.

1.6 Artigo I - Belangero et al., 2014a

Objetivo: Avaliar e comparar pela primeira vez na literatura a expressão dos genes *COL1A1*, *COL1A2*, *COL3A1* e *COL5A1* na região AI (macroscopicamente alterada) e AS (sem alteração pela análise macroscópica) da cápsula glenoumeral de pacientes com IATO.

Casuística: Investigamos um grupo de 18 pacientes com anterior traumática.

Principais resultados: A expressão de *COL1A1*, *COL1A2* e *COL3A1* não diferiu entre as duas regiões do ombro da cápsula. No entanto, observou-se que a expressão de *COL5A1* estava significativamente reduzida na região macroscopicamente afetada.

Conclusão: A região afetada da cápsula glenoumeral em pacientes com IATO apresenta expressão reduzida de *COL5A1*. Esse foi o primeiro trabalho na literatura a investigar expressão gênica em cápsula do ombro e por isso, abriu novas perspectivas de investigação sobre doenças que acometem a cápsula glenoumeral, contribuiu para uma maior compreensão da biologia destas lesões.

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Original Article

Profile of collagen gene expression in the glenohumeral capsule of patients with traumatic anterior instability of the shoulder☆,☆☆

Paulo Santoro Belangero[a,*], *Mariana Ferreira Leal*[a,b], *Alberto de Castro Pochini*[a], *Carlos Vicente Andreoli*[a], *Benno Ejnisman*[a], *Moises Cohen*[a]

[a] *Department of Orthopedics and Traumatology, Federal University of São Paulo (Unifesp), São Paulo, SP, Brazil*
[b] *Department of Morphology and Genetics, Federal University of São Paulo (Unifesp), São Paulo, SP, Brazil*

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ABSTRACT

Objective: To evaluate the expression of the genes *COL1A1*, *COL1A2*, *COL3A1* and *COL5A1* in the glenohumeral capsule of patients with traumatic anterior instability of the shoulder.
Methods: Samples from the glenohumeral capsule of 18 patients with traumatic anterior instability of the shoulder were evaluated. Male patients with a positive grip test and a Bankart lesion seen on magnetic resonance imaging were included. All the patients had suffered more than one episode of shoulder dislocation. Samples were collected from the injured glenohumeral capsule (anteroinferior region) and from the macroscopically unaffected region (anterosuperior region) of each patient. The expression of collagen genes was evaluated using the polymerase chain reaction after reverse transcription with quantitative analysis (qRT-PCR).
Results: The expression of *COL1A1*, *COL1A2* and *COL3A1* did not differ between the two regions of the shoulder capsule. However, it was observed that the expression of *COL5A1* was significantly lower in the anteroinferior region than in the anterosuperior region (median ± interquartile range: 0.057 ± 0.052 *vs.* 0.155 ± 0.398; $p = 0.028$) of the glenohumeral capsule.
Conclusion: The affected region of the glenohumeral capsule in patients with shoulder instability presented reduced expression of *COL5A1*.

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* *Corresponding author.*
E-mail: psbelangero@gmail.com (P.S. Belangero).

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Perfil de expressão de genes do colágeno na cápsula glenoumeral de pacientes com instabilidade traumática anterior do ombro

Palavras-chave:
Instabilidade do ombro
Cápsula articular
Expressão gênica
Matriz extracelular
Colágeno

RESUMO

Objetivo: Avaliar a expressão dos genes *COL1A1*, *COL1A2*, *COL3A1* e *COL5A1* na cápsula glenoumeral de pacientes com instabilidade anterior traumática do ombro.
Métodos: Foram avaliadas amostras de cápsula glenoumeral de 18 pacientes com instabilidade anterior traumática do ombro. Foram incluídos pacientes masculinos, com teste de apreensão positivo e lesão de Bankart no exame de ressonância magnética. Todos os pacientes sofreram mais de um episódio de luxação do ombro. Foram coletadas amostras da cápsula glenoumeral lesionada (região anteroinferior) e da região macroscopicamente não afetada (região anterossuperior) de cada paciente. A expressão dos genes de colágeno foi avaliada por reação em cadeia da polimerase após transcrição reversa com análise quantitativa (qRT-PCR).
Resultados: A expressão de *COL1A1*, *COL1A2* e *COL3A1* não diferiu entre as duas regiões da cápsula do ombro. No entanto, foi observado que a expressão de *COL5A1* estava significantemente reduzida na região anteroinferior em relação à região anterossuperior (mediana ± intervalo interquartílico: 0,057 ± 0,052 *vs* 0,155 ± 0,398; p = 0,028) da cápsula glenoumeral.
Conclusão: A região afetada da cápsula glenoumeral de pacientes com instabilidade do ombro apresentou uma expressão reduzida de *COL5A1*.

Introduction

The great range of motion provided by the scapular belt allows the glenohumeral joint to be used as a stable fulcrum for placing the extremities of the upper limbs in a variety of spatial positions. However, one consequence of this great range of motion is that this joint has a propensity to become unstable.[1]

It is believed that the shoulder is the joint of the human body that most frequently suffers dislocation, with an incidence of 8.2–23.9 cases per 100,000 individuals per year.[2,3] Among these cases, 95% are caused by traumatic events and lesions of the anterior capsule are involved in 90% of these individuals.[4,5] Episodes of shoulder dislocation occur most frequently in young male individuals.[6] Many of the individuals affected practice competitive sports.[7] The recurrence rate for shoulder dislocation is high and reaches up to 100% among young athletes.[8,9]

The anteroinferior (AI) region of the glenohumeral capsule is the location most affected in episodes of traumatic shoulder dislocation.[10] After the first episode of anterior shoulder dislocation, it is common for patients to present shoulder instability.[8,9] Patients with shoulder instability generally present plastic deformation of the capsule, which may result in capsule laxity.[10,11] Previous studies have demonstrated that plastic deformation of the capsule is necessary even in the first dislocation.[12–14] Currently, little is known about the structure of the capsule, especially among patients with shoulder instability. Better comprehension of the underlying biology is important for guiding patient management and for developing new therapeutic options that are complementary to surgery.

The capsule is composed of cellular and fibrous elements. The collagen content of the capsule progressively increases during embryonic development and at birth this tissue is generally fibrous.[15] Types I, III and V fibrillar collagen are the commonest types in the shoulder capsule.[16] Mutations in the genes that code for these collagens have been identified in most of the forms of Ehlers–Danlos syndrome (EDS) and imperfect osteogenesis,[17,18] which present frequent dislocations in several joints, including the shoulder joint. Thus, alterations to these genes may also play a role in shoulder instability.

The aim of the present study was to compare the messenger RNA (mRNA) expression of *COL1A1*, *COL1A2*, *COL3A1* and *COL5A1* between an injured region and another, uninjured region of the glenohumeral capsule in patients with traumatic anterior shoulder instability.

Materials and methods

Patients

Samples were collected from the glenohumeral capsule of 18 patients with traumatic anterior shoulder instability who underwent arthroscopic surgical treatment at Hospital São Paulo, Federal University of São Paulo (UNIFESP), between June 2011 and June 2013. During joint propaedeutics, any presence of associated lesions was noted. All the patients were seen to present capsule redundancy in the anteroinferior region. A free and informed consent statement was obtained from all the patients, as approved by UNIFESP's ethics committee (procedural number: 1085/11). The patients' mean age at the time of the surgery was 30 years (range: 18–42). Their mean

age at the time of the first episode of dislocation had been 25 years (range: 14–37). The mean time that elapsed between the first dislocation and sample collection was 5 years (range: 4 months–10 years).

Only patients with a positive grip test and a Bankart lesion shown on magnetic resonance imaging examination were included in the study. In addition, all the patients reported that they had had two or more episodes of shoulder dislocation. To reduce the heterogeneity of the sample, only male individuals were included. After the first episode of dislocation, the patients were treated with shoulder immobilization for at least two weeks. None of the patients had any history of previous surgery relating to shoulder injuries.

Patients with clinical signs of posterior and/or multidirectional instability and those with generalized hypermobility or hyperlaxity according to Beighton's scale were excluded from the study.[19]

Tissue samples

Tissue samples were collected from two regions of the capsule in each patient: one from the injured region (AI region) and the other from the corresponding macroscopically unaffected region (control), i.e. the anterosuperior (AS) region. All the samples were immediately immersed in RNAlater® solution (Qiagen, Germany) and were then stored at −20 °C until the time of RNA extraction.

Analysis of gene expression

The RNA extraction was performed using the RNeasy® mini-kit (Qiagen, Germany). The concentration and quality were determined using a NanoDrop® spectrophotometer (Kisker, Germany) and the integrity was ascertained by means of electrophoresis on 1% agarose gel. Synthesis of complementary DNA (cDNA) was performed using the high-capacity cDNA archive kit (Life Technologies, USA).

The expression of *COL1A1*, *COL1A2*, *COL3A1* and *COL5A1* was evaluated by means of the polymerase chain reaction after reverse transcription with quantitative analysis (qRT-PCR), using the 7500 Fast Real-Time PCR system (Life Technologies, USA). Os genes *ACTB* and *GAPDH* were selected as internal controls for normalizing the initial quantity of cDNA and correcting possible variations that might affect the efficiency of the qRT-PCR. All qRT-PCR runs were performed in triplicate for all the target genes (*COL1A1*: Hs00164004_mL; *COL1A2*: Hs00164099_mL; *COL3A1*: Hs00943809_mL; and *COL5A1*: Hs00609088_mL) and for the reference genes (β-actin, *ACTB*: 4352935e; and glyceraldehyde-3-phosphate dehydrogenase, *GAPDH*: Hs99999905_mL) through usage of commercially available primers and probes (Life Technologies, USA).

The gene expression was determined in accordance with the formula: ΔCt (*cycle threshold*) = Ct target gene (collagen) – geometric mean of the Ct of the reference genes.[20] The quantification of the expression was adjusted for the amplification efficiency of each gene (*COL1A1* = 92%; *COL1A2* = 97%; *COL3A1* = 103%; *COL5A1* = 94%; *ACTB* = 91%; and *GAPDH* = 92%).

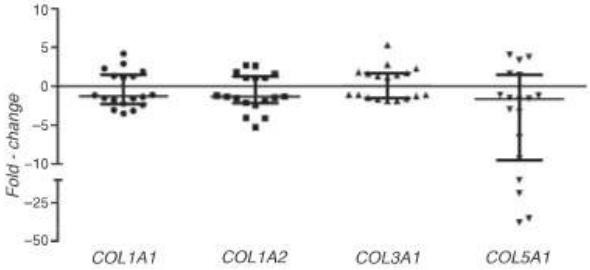

Fig. 1 – Alteration of collagen gene expression in the glenohumeral capsule of patients with shoulder instability. Expression values in the anteroinferior region in relation to the corresponding values in the anterosuperior region of the glenohumeral capsule (fold-change). Horizontal lines indicate the medians and interquartile intervals.

Statistical analysis

The Shapiro–Wilk test was performed to evaluate whether the data presented normal distribution. Since the majority of the variables analyzed did not present normal distribution, the nonparametric Wilcoxon test was used to compare gene expression between pairs of samples from the anteroinferior and anterosuperior regions. The effect size for the Wilcoxon test (r) was calculated in accordance with the formula $r = Z/\sqrt{N}$,[21] in which $r < 0.1$ was considered to be a trivial effect, $0.1 \leq r < 0.3$ small, $0.3 \leq r \leq 0.5$ moderate and $r > 0.5$ large.

Spearman's correlation was used to evaluate whether there was any correlation between collagen gene expression and the age at which the patient was affected, the age at the time of the surgery or the time that had elapsed between the first dislocation and sample collection.

p values <0.05 were considered statistically significant. Medians and interquartile intervals of the data obtained in this study are presented.

Results

The relative expression of the genes *COL1A1*, *COL1A2*, *COL3A1* and *COL5A1* presented wide variation among the individuals with traumatic anterior shoulder instability. Both increased and decreased collagen gene expression in the anteroinferior region, in relation to the anterosuperior region, was observed (Fig. 1). The expression of *COL1A1*, *COL1A2* and *COL3A1* did not differ significantly between the AI and AS regions (Table 1). However, the AI region presented reduced expression of *COL5A1* in relation of the AS region of the glenohumeral capsule of patients with shoulder instability ($p = 0.028$; $r = -0.3665$; Table 1).

No correlation between the levels of collagen gene expression and the age at which the patient was affected, the age at the time of surgery or the time that had elapsed from the first dislocation to sample collection was observed in either of the regions studied ($p > 0.05$ for all the analyses).

Table 1 – Collagen gene expression in the glenohumeral capsule of patients with shoulder instability.

Gene	AI (median ± IQ)	AS (median ± IQ)	*p* value	Effect size (*r*)
COL1A1	1.068 ± 1.597	1.681 ± 1.801	0.372	−0.1488
COL1A2	0.555 ± 0.413	0.650 ± 0.455	0.145	−0.2431
COL3A1	0.716 ± 0.608	0.678 ± 0.662	0.879	−0.0253
COL5A1	0.057 ± 0.052	0.155 ± 0.398	0.028[a]	−0.3665

AI, samples from the anteroinferior region; AS, samples from the anterosuperior region; IQ, interquartile interval.
[a] Statistically significant difference between the anteroinferior and anterosuperior regions, from Wilcoxon analysis, $p < 0.05$.

Discussion

This study demonstrated that patients with anterior shoulder instability present decreased expression of the gene *COL5A1* in the AI region of the glenohumeral capsule, in comparison with the AS region. Type V collagen accounts for 2–5% of the total amount of collagen in most tissues.[22] This type of collagen intercalates with type I collagen, which is the predominant protein in the capsule, to form heterotypic fibrils.[23] Although type V collagen is the fibrillar collagen present in the smallest quantities, it has a central role in regulating fibrillogenesis in the connective tissues.[24] In EDS cases, decreased *COL5A1* expression has been described in around 25–30% of the patients with mutations of this gene.[25] In these patients, formation of abnormal collagen fibrils that are wide and irregular has been observed.[26] In a classical animal model for EDS, decreased *COL5A1* expression was associated with reductions in the numbers of fibrils and with presence of a very large and structurally aberrant subpopulation of fibrils with deficiencies of type V collagen.[24] Deregulation of *COL5A1* expression seems to have a fundamental role in structural alterations to the joint capsule, both in individuals with EDS and in animal models for this syndrome. Thus, we hypothesized that decreased *COL5A1* expression in the AI region of the glenohumeral capsule in patients with shoulder instability could result in disorganized collagen fibrils, thereby contributing towards increased laxity of this tissue in the individuals evaluated.[14,27] On the other hand, decreased *COL5A1* expression in the AI region in relation to the AS region might also be an intrinsic characteristic of the AI region that would contribute towards greater susceptibility of this region to injury, in the patients investigated.

The expression of the genes *COL1A1*, *COL1A2* and *COL3A1* did not differ between the AI and AS regions. However, the number of samples evaluated was a limiting factor in the present study. With the number of samples obtained, our analysis had a power of around 80% for detecting a variation with an effect size of 75% (large effect size), for a type I error of 5%. Large effect sizes were not detected in any of the analyses and therefore the observed power was low. Thus, our analyses had a high likelihood of not detecting a difference between the groups that in reality existed (false negatives). Further studies are needed in order to understand the role of *COL1A1*, *COL1A2* and *COL3A1* in the glenohumeral capsule of patients with shoulder instability.

In the present study, only male patients who had had more than one episode of shoulder dislocation were evaluated. The aim in making this selection was to homogenize our sample. In addition, paired samples with lesions and without lesions (controls) were obtained from the glenohumeral capsule of the same individual, so as to avoid the possibility of bias caused by biological variations between individuals. This is a strategy commonly used in molecular studies that seek to understand the processes of carcinogenesis.[28] However, the mRNA levels of all the collagen genes studied were heterogenous between the patients with shoulder instability. This heterogeneity may have been due to different environmental exposures, such as variations in the number of episodes of dislocation and the time that elapsed between the dislocating events and the surgery. Moreover, like other acute soft-tissue injuries, shoulder instability is a multifactorial disease and therefore intrinsic factors, such as polymorphisms of collagen genes[29,30] or of genes coding for proteins involved in regulating their expression, may also contribute towards heterogeneity between patients and towards greater risk of the disease.

Because the only tissue samples studied were collected during the surgical treatment, it was not possible to assess the regulatory dynamics of gene expression. Nonetheless, it should be noted that this study, to the best of our knowledge, was the first to evaluate gene expression in the glenohumeral capsule of patients with shoulder instability. This study thus contributes towards comprehending this condition.

Conclusions

The injured region of the glenohumeral capsule of patients with shoulder instability presented decreased *COL5A1* expression. Studying gene expression in the glenohumeral capsule of patients with shoulder instability is novel in the literature and opens up new research perspectives regarding this condition. Evaluation of gene expression may contribute towards greater comprehension of the biology of these lesions and thus towards development of new treatment strategies.

Conflicts of interest

The authors declare no conflicts of interest.

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1.7 Artigo II - Belangero et al., 2014b

Objetivo: Avaliar e comparar a expressão dos genes *COL1A1*, *COL1A2*, *COL3A1* e *COL5A1* na cápsula glenoumeral entre pacientes com IATO e indivíduos controles.
Casuística: Foram avaliadas amostras de três regiões da cápsula glenoumeral (região AI, AS e P) de 31 pacientes com IATO e 8 controles.
Principais resultados: A expressão de *COL1A1*, *COL3A1* e a razão *COL1A1/COL1A2* estavam aumentadas em todas as três regiões da cápsula em pacientes comparados aos controles. A expressão de *COL1A2* estava aumentada nas regiões não macroscopicamente afetadas (regiões AS e P). A proporção da expressão de *COL1A2/ COL3A1* estava reduzida em nas regiões AI e P de pacientes em relação aos controles. Na região AI da cápsula de pacientes, as razões *COL1A1/COL5A1*, *CO1A2/COL5A1* e *COL3A1/COL5A1* estavam aumentadas. Em pacientes, *COL1A1/COL5A1* também estava aumentada na região P. A expressão de *COL1A1* e a razão *COL1A1/COL1A2* parece reduzir quanto maior o tempo de sintomas da doença na região AI da cápsula glenoumeral de pacientes com IATO. A expressão de *COL1A1*, *COL3A1* e *COL5A* estava aumentada na região AI de pacientes com um único episódio de deslocamento.
Conclusão: Encontramos desregulação da expressão de genes de colágeno por toda a cápsula de pacientes com instabilidade do ombro. Essas alterações moleculares podem levar a modificações da estrutura das fibrilas do colágeno e comprometimento do processo de cicatrização, possivelmente com um papel na deformação capsular. Essas alterações moleculares podem surgir como resultado da luxação do ombro e pode contribuir para episódios de luxação recorrente.

Gene Expression Analysis in Patients With Traumatic Anterior Shoulder Instability Suggests Deregulation of Collagen Genes

Paulo Santoro Belangero,[1] Mariana Ferreira Leal,[1,2] Eduardo Antônio Figueiredo,[2] Carina Cohen,[1] Alberto de Castro Pochini,[1] Marília Cardoso Smith,[2] Carlos Vicente Andreoli,[1] Sintia Iole Belangero,[2,3] Benno Ejnisman,[1] Moises Cohen[1]

[1]Departamento de Ortopedia e Traumatologia, Universidade Federal de São Paulo, Rua Borges Lagoa, 783 CEP: 04038-031, São Paulo, SP, Brazil, [2]Disciplina de Genética, Departamento de Morfologia e Genética, Universidade Ferderal de São Paulo, 04023-001, São Paulo, SP, Brazil, [3]Laboratório Interdisciplinar de Neurociências Clínicas, Departamento de Psiquiatria, Universidade Federal de São Paulo, 04039-032, São Paulo, SP, Brazil

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ABSTRACT: Shoulder dislocation occurs in 1–2% of the population. Capsular deformation is a key factor in shoulder dislocation; however, little is known about capsule biology. We evaluated, for the first time in literature, the expression of *COL1A1*, *COL1A2*, *COL3A1* and *COL5A1* in the antero-inferior, antero-superior and posterior regions of the glenohumeral capsule of 31 patients with anterior shoulder instability and eight controls. The expression of collagen genes was evaluated by quantitative reverse transcription-PCR. The expression of *COL1A1*, *COL3A1* and the ratio of *COL1A1*/*COL1A2* were increased in all three portions of the capsule in patients compared to controls ($p < 0.05$). *COL1A2* expression was upregulated in the antero-superior and posterior sites of the capsule of patients ($p < 0.05$). The ratio of *COL1A2*/*COL3A1* expression was reduced in capsule antero-inferior and posterior sites of patients compared to controls ($p < 0.05$). In the capsule antero-inferior site of patients, the ratios of *COL1A1*/*COL5A1*, *CO1A2*/*COL5A1* and *COL3A1*/*COL5A1* expression were increased ($p < 0.05$). In patients, *COL1A1*/*COL5A1* was also increased in the posterior site ($p < 0.05$). We found deregulated expression of collagen genes across the capsule of shoulder instability patients. These molecular alterations may lead to modifications of collagen fibril structure and impairment of the healing process, possibly with a role in capsular deformation. © 2014 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 32:1311–1316, 2014.

Keywords: shoulder instability; glenohumeral capsule; gene expression; extracellular matrix; collagen

Shoulder dislocation occurs in 1–2% of the population.[1] Traumatic injuries account for 95% of shoulder dislocation episodes.[2] These shoulder injuries are frequently observed in young athletes involved in competitive sports.[3] In addition, after a first episode of anterior shoulder dislocation, shoulder instability is frequently observed and the recurrence rate is up to 100% in young athletes.[4,5]

The anterior glenohumeral joint capsule is affected in 90% of shoulder dislocations.[6] After a traumatic shoulder dislocation, patients present a plastic deformation of the capsule, which results in capsular laxity.[7,8] The antero-inferior (AI) region of the capsule is the site most often injured.[8,9] AI capsular deformation is described as the real pathogenic pattern of the shoulder dislocation.[10] However, little is known about the capsule biology, especially in patients with shoulder instability. An improved understanding of the underlying biology is important to guide patient management and to develop new therapeutic options complementary to surgery.

The capsule is composed of cellular and fibrous elements. The collagenous content of the capsule progressively increases during development and it is generally fibrous at full term.[11] Type I, III, and V fibrillar collagens are the most common types present in the shoulder capsule.[12] Mutations in genes encoding the collagens have been identified in most forms of Ehlers–Danlos syndrome (EDS) and osteogenesis imperfecta,[13–15] which present frequent joint dislocations, including dislocations of the shoulder. Thus, alterations in these genes may also play a role in shoulder instability.

We hypothesized that gene expression alterations may arise as a result of shoulder dislocation and might be a contributor to recurrent dislocation episodes. Therefore, we evaluated, for the first time in literature, the expression of *COL1A1*, *COL1A2*, *COL3A1*, and *COL5A1* mRNA in three regions of the glenohumeral capsule in patients with traumatic anterior shoulder instability and controls.

METHODS

Patients

We evaluated 31 outpatients with traumatic anterior shoulder dislocation from São Paulo Hospital of the Federal University of São Paulo (UNIFESP), Brazil. All patients were treated for at least 2 weeks with shoulder immobilization after the first episode of shoulder dislocation and underwent arthroscopic surgical treatment for shoulder instability. The following inclusion criteria were employed: positive apprehension test, a Bankart lesion on magnetic resonance imaging and no history of previous surgery for an injured shoulder. Patients with clinical signs of posterior and/or multidirectional instability or presenting generalized joint hyperlaxity or hypermobility by Beighton score[16] were excluded. Moreover, patients with associated lesions, such as superior lesion anterior posterior (SLAP) lesions detected during the surgery, were excluded.

In addition, eight subjects who underwent arthroscopically assisted treatment for acromioclavicular dislocation were included as a control group. These patients did not

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Correspondence to: Mariana Ferreira Leal (T: +55-11-55716621; F: +55-11-55716621; E-mail: mariana.morf@epm.br)

present any history of shoulder instability or positive signs for this injury under anesthesia. Moreover, we did not find any radiological indications of glenohumeral capsule alterations. All controls were physically active.

Informed consent and the approval of the ethics committee of the UNIFESP were obtained (approval number: CAAE 01609812.9.0000.5505) from all patients before data and sample collection. A preoperative questionnaire was given to all patients that included questions regarding demographics, age of onset, number of luxation episodes, duration of symptoms, physical activity, type of work and other clinical variables.

Tissue Samples

During the arthroscopic procedure, tissue samples were obtained from three sites of the glenohumeral capsule of each patient: AI, antero-superior (AS) and posterior (P) sites. To minimize the variation of sampling, the tissue specimens were taken by two of the authors (P.S.B. and B.E.) at the same anatomic location described below. The biopsy samples of AI and AS sites were obtained with the scope in the posterior portal and the basket grasper in the anterior portal. The AI specimen was taken from the most inferior region of glenohumeral capsule next to the inferior glenohumeral ligament. The AS specimen was taken in the direction of the anterior portal, below the biceps tendon, in the rotator interval area. The P specimen was obtained during the evaluation of the posterior capsulolabral complex with the scope in the anterior portal and the basket grasper in the posterior portal. The P sample was taken in the direction of the posterior portal. All tissue specimens were immediately immersed in RNAlater® solution (Qiagen, Hilden, Germany) and then stored at −20°C until RNA extraction.

mRNA Expression Analysis

Total RNA was extracted with an RNeasy® mini kit (Qiagen) following the manufacturer's instructions. RNA concentration and quality were determined using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, DE), and RNA integrity was verified by 1% agarose gel electrophoresis. cDNA was synthesized using a High-Capacity cDNA Archive kit (Life Technologies, Carlsbad, CA). *COL1A1*, *COL1A2*, *COL3A1*, and *COL5A1* expression was evaluated by quantitative reverse transcription PCR (qRT-PCR) using a 7500 Fast Real-Time PCR System (Life Technologies), with TaqMan® primers and probes purchased as Assays-on-demand Products for Gene Expression (Life Technologies). The *ACTB* and *GAPDH* genes were selected as internal controls to normalize the sample input amount. All qRT-PCR reactions were performed in triplicate for all target genes (*COL1A1*: Hs00164004_m1; *COL1A2*: Hs00164099_m1; *COL3A1*: Hs00943809_m1; *COL5A1*: Hs00609088_m1) and reference genes (*ACTB*: 4352935e; *GAPDH*: Hs99999905_m1). Gene expression was normalized to the geometric mean of reference genes [ΔCt (cycle threshold) = target gene (collagen) Ct—mean of reference genes Ct]. The abundance of mRNA expression was adjusted according to amplification efficiency.[17]

Statistical Analysis

We verified the distribution of all continuous variables using the Shapiro–Wilk normality test to determine the appropriate tests for subsequent statistical comparisons. The expression data were not normally distributed. Therefore, the Mann–Whitney test was performed to compare the gene expression between the studied groups and clinical variables. A Chi-square test was used to compare the gender distribution between cases and controls. Spearman's correlation was applied to evaluate the possible correlation between gene expression and age at surgery or age of onset. A p-value of <0.05 was considered statistically significant. All values are shown as the median ± interquartile range.

RESULTS

Patient Data and Clinical Outcomes

Table 1 shows the clinical outcomes of the shoulder instability patients. In our experimental group, 56.5% of shoulder instability patients with less than 1 year of the condition (time between the onset and surgery) presented 2 or more dislocation episodes, whereas all patients with more than 1 year of the condition presented 2 or more dislocation episodes. Therefore, the number of dislocation episodes was significantly higher in patients with more than one year of the condition ($p = 0.006$). Although no histological assessment was performed, a macroscopic evaluation during the arthroscopic procedure revealed that all shoulder instability patients presented a more flexible capsular aspect in the AI site (Fig. 1).

Among controls, 7 (87.5%) were males and 1 (12.5%) was female, and the mean age at time of surgery was 32.5 ± 11.7 years. No significant difference was observed in the distribution of gender between groups ($p = 1$). In addition, the age at the time of surgery was not significantly different between cases and controls ($p = 0.306$).

Differences Between Cases and Controls

Table 2 shows the mean and standard deviations of *COL1A1*, *COL1A2*, *COL3A*, and *COL5A1* expression, as well as ratios of expression, in the AI, AS, and P

Table 1. Distribution of the Clinical Outcomes of Shoulder Instability Patients

Variable	Distribution
Age at surgery, years (mean ± SD)	28.77 ± 7.22
Age of onset, years (mean ± SD)	25.47 ± 6.75
Gender [*N* (%)]	
Male	28 (90.3)
Female	3 (9.7)
Duration of condition, years (mean ± SD)	3.23 ± 5.47
Duration of condition [*N* (%)]	
≤1 year	18 (58.1)
>1 year	13 (41.9)
Number of injuries [*N* (%)]	
1 dislocation	8 (25.8)
>1 dislocation	23 (74.2)
Type of sport [*N* (%)]	
No sport	5 (16.1)
Noncontact	8 (25.8)
Contact	18 (58.1)
Manual job [*N* (%)]	
No	22 (71)
Yes	9 (29)

N, number of patients; SD, standard deviation.

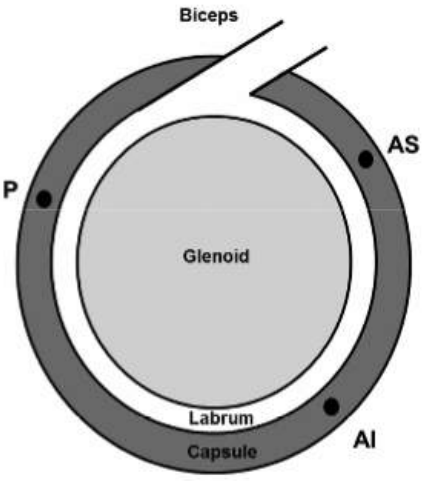

Figure 1. Representative biopsy sites in the glenohumeral capsule. Dots show the portion of the capsule where samples were obtained. AI, antero-inferior portion; AS, antero-superior portion; P, posterior portion.

sites of the glenohumeral capsule of cases and controls. Shoulder instability patients presented increased *COL1A1* ($p = 0.003$, for AI site; $p < 0.001$, for AS site; $p < 0.001$, for P site) and *COL3A1* ($p = 0.005$, for AI site; $p = 0.002$, for AS site; $p = 0.001$, for P site) expression in all three portions of the capsule compared to controls. However, the expression of *COL1A2* was upregulated in the AS ($p = 0.010$) and P ($p = 0.001$) sites of the capsule of patients compared to controls. *COL5A1* expression was not significantly different between cases and controls in the three portions of the shoulder capsule examined ($p > 0.05$).

In addition, the expression ratio of *COL1A1*/*COL1A2* mRNA was increased in all three sites of the glenohumeral capsule of shoulder instability patients compared to controls ($p < 0.001$, for the AI site; $p = 0.003$, for the AS site; $p = 0.008$, for the P site). In the AI site of the capsule of patients, *COL1A1*/*COL5A1* ($p < 001$), *CO1A2*/*COL5A1* ($p = 0.012$) and *COL3A1*/*COL5A1* ($p = 0.002$) expression was significantly increased. *COL1A1*/*COL5A1* expression was also significantly increased in the P site ($p = 0.019$). In contrast, a reduced ratio of *COL1A2*/*COL3A1* expression was detected in the AI ($p = 0.003$) and P ($p = 0.044$) sites of the capsule of studied patients compared to controls.

Clinical Outcomes and Collagen Expression

Regarding the number of injuries, *COL1A1* (3.624 ± 3.18 vs. 1.048 ± 0.68, $p = 0.21$; Fig. 3A), *COL3A1* (1.099 ± 0.10 vs. 0.635 ± 0.27, $p = 0.024$; Fig. 3B) and *COL5A1* (0.146 ± 1.49 vs. 0.054 ± 0.06, $p = 0.011$; Fig. 3C) expression was increased in the AI capsule portion of patients with a single episode compared to patients with recurrent shoulder dislocation. Moreover, patients with less than one 1 year of shoulder instability symptoms demonstrated increased *COL1A1* (2.039 ± 4.875 vs.

Table 2. Collagen Gene Expression in the Glenohumeral Capsule of Patients With Shoulder Instability and Controls

	AI			AS			P		
Gene	Cases (Median ± IQR)	Controls (Median ± IQR)	*p*-Value	Cases (Median ± IQR)	Controls (Median ± IQR)	*P*-Value	Cases (Median ± IQR)	Controls (Median ± IQR)	*p*-Value
COL1A1	1.334 ± 3.16	0.312 ± 0.44	0.003*	1.672 ± 1.98	0.386 ± 0.49	<0.001*	1.07 ± 1.97	0.230 ± 0.15	<0.001*
COL1A2	0.613 ± 0.45	0.416 ± 0.428	0.401	0.599 ± 0.50	0.302 ± 0.33	0.010*	0.482 ± 0.354	0.231 ± 0.21	0.001*
COL3A1	0.800 ± 0.69	0.255 ± 0.46	0.005*	0.604 ± 0.69	0.191 ± 1.38	0.002*	0.464 ± 0.72	0.167 ± 0.21	0.001*
COL5A1	0.074 ± 0.09	0.155 ± 0.21	0.184	0.165 ± 0.41	0.104 ± 0.11	0.279	0.197 ± 0.35	0.095 ± 0.09	0.184
COL1A1/*COL1A2*	2.741 ± 3.55	0.760 ± 0.68	<0.001*	2.105 ± 2.15	0.960 ± 0.77	0.003*	2.230 ± 1.18	1.259 ± 0.77	0.008*
COL1A1/*COL3A1*	1.877 ± 1.86	0.995 ± 3.74	0.067	2.57 ± 1.89	1.571 ± 2.39	0.162	1.787 ± 3.20	1.996 ± 2.22	0.878
COL1A1/*COL5A1*	17.379 ± 51.86	2.100 ± 2.01	<0.001*	4.98 ± 19.50	3.016 ± 3.06	0.107	6.483 ± 10.08	2.523 ± 1.26	0.019*
COL1A2/*COL3A1*	0.724 ± 0.48	1.707 ± 3.47	0.003*	0.976 ± 1.31	2.104 ± 1.17	0.132	0.988 ± 1.23	1.774 ± 1.59	0.044*
COL1A2/*COL5A1*	6.779 ± 6.94	3.129 ± 2.08	0.012*	2.911 ± 8.18	3.472 ± 3.56	0.932	3.28 ± 5.05	2.050 ± 3.42	0.311
COL3A1/*COL5A1*	9.574 ± 18.04	1.84 ± 1.90	0.002*	2.185 ± 14.18	2.034 ± 2.92	0.505	3.877 ± 6.92	1.132 ± 2.22	0.099

AI, antero-inferior portion of the glenohumeral capsule; AS, antero-superior portion of the glenohumeral capsule; P, posterior portion of the glenohumeral capsule; IQR, interquartile range. *Significant difference between groups by Mann–Whitney test ($p < 0.05$).

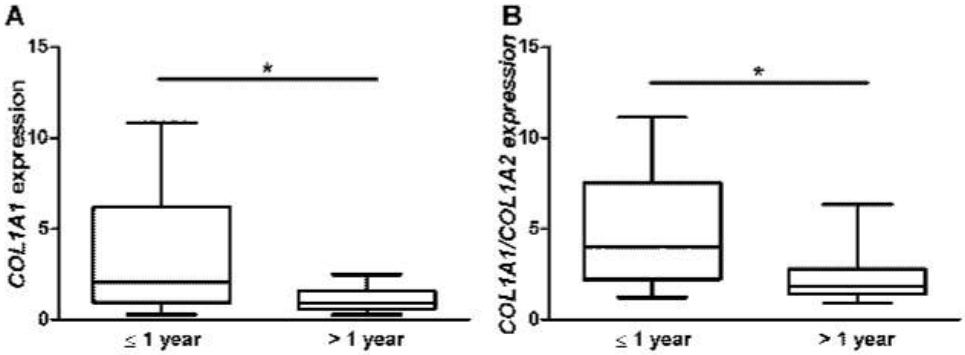

Figure 2. Collagen expression by time of shoulder instability symptoms. Increased *COL1A1* (A) and *COL1A1*/*COL1A2* (B) expression in the antero-inferior site of the glenohumeral capsule of shoulder instability patients with ≤1 year of symptoms compared to patients with >1 year of symptoms. *p-value <0.05 by Mann–Whitney test.

0.918 ± 0.763, $p = 0.022$; Fig. 2A) and *COL1A1*/*COL1A2* (4.015 ± 4.553 vs. 1.82 ± 1.25, $p = 0.009$; Fig. 2B) expression in the AI region compared to patients with more than 1 year of symptoms. No association between collagen gene expression, ratios of gene expression or any other clinical variable was found in shoulder instability patients ($p > 0.05$).

DISCUSSION

The AI portion of the glenohumeral capsule of shoulder instability patients commonly exhibits macroscopic alteration,[7,8] such as the capsular deformation that was detected during surgical treatment in all the studied patients. Previously, a macroscopic analysis of the collagen fiber bundle architecture in the AI region of the glenohumeral capsule revealed that a system of bundles spirally crossing one another permits the entire capsule to resist tensile and shear loads.[18] Therefore, there is a reciprocal load-sharing relationship in the capsule, whereby tensile load in either the anterior or superior structures is simultaneously accompanied by laxity in the P or inferior portion, respectively.[8]

In the present study, we found deregulated expression of collagen genes in all regions (AI, AS, and P) of the capsule of shoulder instability patients. Therefore, our investigation demonstrated that anterior shoulder dislocation might lead to molecular alterations across the capsule even in patients without multidirectional instability.

Here, we observed that *COL1A1* and *COL3A1* expression was increased in three sites of the glenohumeral capsule of shoulder instability patients compared to controls. Moreover, *COL1A2* were also upregulated in the AS and P sites of the capsule of shoulder instability patients. Upregulation of these genes or their protein products has been reported in several joint injuries, including injured Achilles tendon,[19,20] anterior cruciate ligament[21–23] and rotator cuff tear.[24,25] COL1 is the most prominent protein of the capsule, as well as of ligaments and tendons, and the primary protein responsible for resisting physiological loads for different activities. However, COL3, with its ability to form extensive crosslinks, seems to modulate the growth in the diameter of the collagen fibrils.[26,27] During joint healing, COL3 is postulated to form the architecture of an early repair construct, which is then infiltrated and replaced with COL1.[28] In tendons, it has been suggested that the ratio of *COL1*/*COL3* may be an indicator of total repair response, with the early increase of *COL3* initiating the repair, the later increase of *COL1* reflecting maturation, and the return to baseline ratio level indicating conclusion of the repair process.[28] Therefore, we hypothesize that the repair process of capsule tissue of the shoulder instability patients was still incomplete.

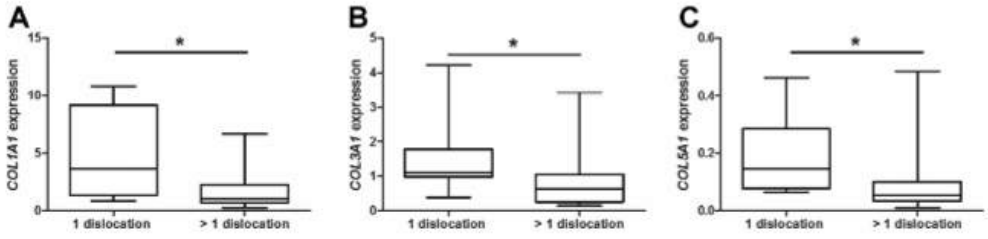

Figure 3. Collagen expression by number of dislocation episodes. Increased *COL1A1* (A), *COL3A1* (B), and *COL5A1* (C) expression in the antero-inferior site of the glenohumeral capsule of shoulder instability patients with one dislocation episode compared to patients with more than 1 dislocation episode. *p-value <0.05 by Mann–Whitney test.

In the AI site, *COL1A1* expression was upregulated in patients with less than 1 year of the condition compared to patients with more than 1 year of condition. Moreover, *COL1A1*, *COL3A1*, and *COL5A1* expression was upregulated in AI site of the capsule of patients with a single episode of shoulder dislocation. Therefore, our results suggest that collagen gene expression deregulation may arise as a result of the first shoulder dislocation episode. However, we hypothesize that the regulation of collagen gene expression in the capsule tissue is dynamic and, over time, the expression of these genes is continuously modulated in an attempt to reach a state of balance. Our findings suggest that the collagen expression alterations might be a contributor to recurrent dislocation episodes.

In this study, we detected an increased ratio of *COL1A1*/*COL1A2* in all three sites of the capsule of the studied patients compared to controls. The COL1 fibril is usually a heterotrimer of α1 chains and α2 chains at a ratio of 2:1. However, homotrimers consisting of three α1 chains have been reported in fetal tissues, genetic disorders associated with α2 (I) chain deficiency, fibrotic tissues, carcinomas and fetal and cancer cell cultures.[29] Here, we hypothesize that the increased ratio of *COL1A1*/*COL1A2* expression may facilitate the formation of COL1 homotrimers in the glenohumeral capsule of shoulder instability patients. Based on electron microscopy data, the COL1 homotrimers appear to be more narrow and aligned in a disorganized manner.[30] In addition, the COL1 homotrimers are resistant to the collagenases of the matrix metalloproteinase.[31] A previous study suggested that even a minor fraction of the homotrimers might alter tissue remodeling by resulting in a pathogenic accumulation of collagenase-resistant fibers.[29] Therefore, increased *COL1A1* expression in the glenohumeral capsule may lead to unbalanced collagen homeostasis, thereby affecting normal tissue structure and the healing process. Moreover, as for *COL1A1*, the increased *COL1A1*/*COL1A2* expression especially in the AI region of patients with less than 1 year of the condition also suggests that this macroscopically affected portion of the capsule presents unbalanced collagen expression probably due to incomplete healing process.

In the present study, we also demonstrated that the ratio of *COL1A1*/*COL5A1*, *COL1A2*/*COL5A1*, and *COL3A1*/*COL5A1* was increased in the AI region of the glenohumeral capsule of shoulder instability patients. In the P site, *COL1A1*/*COL5A1* was also increased. These findings may be in part due to the increased expression of *COL1A1*, *COL1A2*, and *COL3A1* in the patients. However, it is important to highlight that *COL5A1* expression was associated with the number of dislocations episodes. This collagen co-assembles with COL1 as heterotypic fibrils[32] and plays a central role in the regulation of fibrillogenesis.[33,34] In mouse models, down-regulation of *Col5a1* mRNA expression was associated with a reduction in fibril number and the presence of a very large and structurally aberrant subpopulation of fibrils that lacked type V collagen.[33] Moreover, these abnormal fibrils disrupted the normal linear and lateral growth mediated by fibril fusion.[33] Further investigations are still necessary to understand the *COL5A1* role in the shoulder instability.

To our knowledge, this is the first study to detect gene expression alterations resulting from dislocation episodes in the glenohumeral capsule. However, this study has some limitations. First, we evaluated a single time point (at the time of surgical repair) thus, we were unable to evaluate the dynamic regulation of gene expression. Moreover, some statistical analyses exhibited reduced power to detect significant differences between groups, and this was most likely due to the high degree of heterogeneity among patients with anterior shoulder instability. Therefore, false-negative results may have occurred.

In conclusion, we found deregulated expression of collagen genes across the capsule of patients with shoulder dislocations. An imbalance in the expression ratio of several collagen genes, particularly in the AI site, was detected. These molecular alterations may lead to modifications of collagen fibril structure and of the tissue healing process, as well as to capsular deformation. Therefore, *COL1A1*, *COL1A2*, *COL3A1*, and *COL5A1* may play a role in shoulder instability.

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1.8 Artigo III - Belangero et al., 2016a

Objetivo: avaliar o papel da expressão dos genes *TGFB1*, *TGFBR1*, *LOX*, *PLOD1* e *PLOD2* (relacionados ao processo de *crosslink* do colágeno) na IATO.

Casuística: Foram avaliadas amostras de três regiões da cápsula glenoumeral (região AI, AS e P) de 31 pacientes com IATO e 8 controles.

Principais resultados: Observamos que a expressão de *PLOD2* estava aumentada na região AI dos doentes em comparação com os controles. Houve uma tendência de aumento de *LOX* na porção P de pacientes. Pacientes com luxação do ombro recorrente apresentaram maior expressão de *TGFBR1* na parte cápsula AI e de *PLOD2* na região P. Por outro lado, *LOX* estava aumentado na porção P da cápsula de pacientes com um único episódio de deslocamento de ombro. Na região AI, a expressão de *LOX* estava inversamente correlacionada, enquanto a expressão de *TGFBR1* estava diretamente correlacionada com a duração dos sintomas. Na região P, *PLOD2*, *TGFB1* e *TGFBR1* estavam diretamente correlacionados com a duração dos sintomas.

Conclusão: A expressão *PLOD2* estava aumentada na região macroscopicamente afetada da cápsula de pacientes. O aumento da expressão de *TGFB1*, *TGFBR1* e *PLOD2* parece estar relacionado com a duração dos sintomas da doença, especialmente na região P. O aumento da expressão de *LOX* parece ocorrer apenas na fase inicial da afecção. Portanto, *TGFB1*, *TGFBR1*, *LOX* e *PLOD2* podem desempenhar um papel na instabilidade do ombro.

Expression Analysis of Genes Involved in Collagen Cross-Linking and Its Regulation in Traumatic Anterior Shoulder Instability

Paulo Santoro Belangero,[1] Mariana Ferreira Leal,[1,2] Carina Cohen,[1] Eduardo Antônio Figueiredo,[2] Marília Cardoso Smith,[2] Carlos Vicente Andreoli,[1] Alberto de Castro Pochini,[1] Benno Ejnisman,[1] Moises Cohen[1]

[1]Departamento de Ortopedia e Traumatologia, Universidade Federal de São Paulo, São Paulo, São Paulo 04038-031, Brazil, [2]Disciplina de Genética, Departamento de Morfologia e Genética, Universidade Federal de São Paulo, São Paulo, São Paulo 04023-001, Brazil

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ABSTRACT: The molecular alterations involved in the capsule deformation presented in shoulder instability patients are poorly understood. Increased TGFβ1 acts as a signal for production of matrix macromolecules by fibrogenic cells at joint injury sites. TGFβ1, through its receptor TGFβR1, regulates genes involved in collagen cross-linking, such as LOX, PLOD1, and PLOD2. We evaluated *TGFβ1*, *TGFβR1*, *LOX*,*PLOD1*, and *PLOD2* gene expression in the antero-inferior (macroscopically injured region), antero-superior and posterior regions of the glenohumeral capsule of 29 shoulder instability patients and eight controls. We observed that *PLOD2* expression was increased in the anterior-inferior capsule region of the patients compared to controls. *LOX* expression tended to be increased in the posterior portion of patients. Patients with recurrent shoulder dislocation presented upregulation of *TGFβR1* in the antero-inferior capsule portion and of *PLOD2* in the posterior region. Conversely, *LOX* was increased in the posterior portion of the capsule of patients with a single shoulder dislocation episode. In the antero-inferior, *LOX* expression was inversely correlated and *TGFβR1* was directly correlated with the duration of symptoms. In the posterior region, *PLOD2*, *TGFβ1*, and *TGFβR1* were directly correlated with the duration of symptoms. In conclusion, *PLOD2* expression was increased in the macroscopically injured region of the capsule of patients. Upregulation of *TGFβ1*, *TGFβR1*, and *PLOD2* seems to be related with the maintenance of disease symptoms, especially in the posterior region. *LOX* upregulation seems to occur only in the initial phase of the affection. Therefore, *TGFβ1*, *TGFβR1*, *LOX*, and *PLOD2* may play a role in shoulder instability. © 2015 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 34:510–517, 2016.

Keywords: shoulder instability; gene expression; TGFβ1 signaling; collagen cross-linking

Shoulder dislocation is a common reason for emergency room visits and accounts for about 45% of all dislocations.[1] Traumatic shoulder dislocations are far more common than intentional and/or non-traumatic forms, which are managed by rehabilitation therapy and are not considered herein. Anterior shoulder dislocations contribute 96–98% of all shoulder dislocations.[1] The incidence of first-time anterior shoulder dislocation ranges from 8 to 8.2/100,000 population/year and the prevalence is about 2%.[1] Shoulder instability (SI) is often observed after the initial episode of shoulder dislocation, with a recurrence rate of up to 100% in young athletes.[2,3]

Wound healing is a complex process that requires deposition and accumulation of newly synthesized structural proteins as well as degradation of old or damaged structures composed mainly of the extracellular matrix (ECM).[4] In a previous study, we observed deregulated expression of collagen genes across the capsule of patients with traumatic anterior shoulder instability.[5] The expression of collagen genes were increased in the antero-inferior (AI), antero-superior (AS), and posterior (P) portions of the capsule in patients compared to controls. These molecular alterations may have a role in collagen fibril structure and in the tissue healing process.

An inflammatory response commonly occurs in the earliest phase of wound healing, followed by new connective tissue matrix deposition.[6] Increases of transforming growth factor β1 (TGFβ1) accompany the acute inflammatory phase and appear to act as a signal modulating the production of matrix macromolecules by fibrogenic cells at the injury site.[7] The TGFβ is activated by proteolytic cleavage.[8] This activity is mediated by 2 signaling receptors, TGFβ receptor I (TGFβ1) and TGFβ receptor 2 (TGFβR2), which dimerize and transduce their signal via their serine threonine kinase activity.[9] TGFBR1 is the central propagator of TGFβ signaling.[10]

In the shoulder capsule of patients with adhesive capsulitis, TGFβ was associated with fibrosis and accumulation of a dense matrix of type I and type III collagen within the capsule.[11,12] Moreover, several studies reported similar molecular alterations in adhesive capsulitis and Dupuytren disease. In Dupuytren disease, the increased expression of TGFβ1 and its receptor TGFβR1 has previously been described.[13,14]

TGFβ1 regulates important collagen-modifying enzymes, such as the lysyl oxidase (LOX)[15] and lysyl hydroxylases 1 and 2 (encoded by *PLOD1* e *PLOD2* genes, respectively).[16–18] LOX plays a key role in the maturation of the ECM. LOX is a secreted, copper-dependent amine oxidase which plays a substantial role in the biogenesis of the connective tissue matrix by oxidizing lysine residues in elastin and collagen, thereby initiating the formation of covalent cross-links

Paulo Santoro Belangero and Mariana Ferreira Leal contributed equally to this study.
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Correspondence to: Mariana Ferreira Leal (T: +55-11-55716621; F: +55-11-55716621; E-mail: mariana.morf@epm.br

which stabilize these fibrous proteins.[19,20] LOX activity is essential to maintain the tensile and elastic features of connective tissues.[20,21] In many pathological fibrotic situations, the expression of the cross-linked enzyme LOX and its enzymatic activity are controlled by TGFβ1.[15] Additionally, differential variations of TGFβ1 were able to induce the LOX activity in an in vitro model of mechanical injury in ligament cells.[22]

The lysyl hydroxylases promote ECM structural stability and maturation by promoting inter- and intramolecular cross-links and the addition of carbohydrate moieties to ECM molecules.[23,24] Patients with Ehlers–Danlos Syndrome (EDS) type VI present mutations in the *PLOD1* gene, which result in reduced activity of this lysyl hydroxylase. EDS type six is a heritable disorder characterized by kyphoscoliosis, joint laxity, skin fragility, and muscle hypertonia.[25] Therefore, PLOD1 has an important role in joint tissue structure and function. PLOD2 is implicated in several pathological processes, including fibrosis. The increased expression of *PLOD2* was described in fibroblasts isolated from keloid, hypertrophic scars, and the *palmar fascia* of patients with Dupuytren disease.[26] Furthermore, some patients with EDS also present reduced *PLOD2* expression in skin fibroblasts.[27] To our knowledge, no lysyl oxidase, or hydroxylase has been implicated in normal or pathological processes in the glenohumeral capsule.

We hypothesized that gene expression alterations may arise as a result of shoulder dislocation and might be a contributor to recurrent dislocation episodes. We therefore evaluated, for the first time in literature, the expression of *TGFβ1, TGFβR1, LOX, PLOD1*, and *PLOD2* mRNA in three regions of the glenohumeral capsule in patients with traumatic anterior shoulder instability and controls. Moreover, we investigated the possible associations between gene expression in capsule samples and preoperative clinical parameters.

METHODS

This is a case-control study (level of evidence three) in which samples of patients with and without shoulder instability were evaluated.

Patients

We evaluated 29 outpatients with traumatic anterior shoulder dislocation from São Paulo Hospital of the Federal University of São Paulo (UNIFESP), Brazil. All patients were treated for at least 2 weeks with shoulder immobilization after the first episode of shoulder dislocation and underwent arthroscopic surgical treatment for shoulder instability. The following inclusion criteria were employed: positive apprehension test, a Bankart lesion on magnetic resonance imaging and no history of previous surgery for an injured shoulder. Patients with clinical signs of posterior and/or multidirectional instability or presenting generalized joint hyperlaxity or hypermobility by Beighton score[28] were excluded. Moreover, patients with associated lesions, such as superior lesion anterior posterior (SLAP) lesions detected during the surgery, were excluded.

In addition, eight subjects who underwent arthroscopically assisted treatment for acromioclavicular dislocation were included as a control group. These patients did not present any history of shoulder instability or positive signs for this injury under anesthesia. Moreover, we did not find any radiological indications of glenohumeral capsule alterations. A standard complete joint examination with scope and probe during arthroscopy confirmed that the controls did not present any other concomitant pathology in the shoulder. All controls were physically active.

Informed consent and the approval of the ethics committee of the UNIFESP were obtained (approval number: CEP 51436) from all patients before data and sample collection. A preoperative questionnaire was given to all patients that included questions regarding demographics, age of onset, number of luxation episodes, duration of symptoms, physical activity, type of work, and other clinical variables. In our sample, physical activity involving the upper limbs included basketball, handball, tennis, swimming, climbing, and the some martial arts (judo, jiu–jitsu and capoeira, a Brazilian martial art).

Tissue Samples

During the arthroscopic procedure, tissue samples were obtained from three sites of the glenohumeral capsule of each patient: AI, AS, and P sites. To minimize the variation of sampling, the tissue specimens were taken by two of the authors (PSB and BE).

The samples were collected as previously described by our group.[29] The biopsy samples of AI and AS sites were obtained with the scope in the posterior portal and the basket grasper in the anterior portal. The AI specimen was taken from the most inferior region of the glenohumeral capsule next to the inferior glenohumeral ligament. The AS specimen was taken in the direction of the anterior portal, below the biceps tendon, in the rotator interval area. The P specimen was obtained during the evaluation of the posterior capsulolabral complex with the scope in the anterior portal and the basket grasper in the posterior portal. The P sample was taken in the direction of the posterior portal.

All tissue specimens were immediately immersed in RNAlater® solution (Qiagen, Germany) and then stored at −20°C until RNA extraction.

RNA Extraction

Total RNA was extracted with an RNeasy® mini kit (Qiagen, Germany) following the manufacturer's instructions. The mechanical lysis step was performed using the Tissue Lyser LT equipment (Qiagen, Germany). RNA concentration and quality were determined using a NanoDrop ND-1,000 spectrophotometer (Thermo Scientifc, Wilmington, DE), and RNA integrity was verified by 1% agarose gel electrophoresis. Aliquots of the total RNA were stored at −80°C until further use.

mRNA Expression Analysis

Gene expression was evaluated by reverse-transcription quantitative polymerase chain reaction (RT-qPCR), which is currently considered to be the gold standard technique for the analysis of mRNA level.[30] First, cDNA was synthesized using a High-Capacity cDNA Archive kit (Life Technologies, Foster City, CA) according to the manufacturer's protocol.

To detect the range of expression of the studied genes, reactions were performed with 80–100 ng of cDNA input using TaqMan Low-Density Array (TLDA) cards (Life Technologies, Foster City, CA) and ViiA 7 Real-Time PCR System (Life Technologies, Foster City, CA). Only inventoried TaqMan Gene Expression Assays (Life Technologies, Foster City, CA) were chosen for gene expression analysis. The final volume in each TLDA well is approximately 1 μl.

The *HPRT1* and *B2M* genes were used as internal controls to normalize the sample input amount, based on our previous study that identified suitable reference genes for the gene expression studies in glenohumeral capsule.[31] All qRT-PCR reactions were performed in triplicate for all target genes (*LOX*: Hs00942480_m1; *PLOD1*: Hs00609368_m1; *PLOD2*: Hs01118190_m1; *TGFβ1*: Hs00998133_m1; *TGFβR1*: Hs00610320_m1) and reference genes (*HPRT1*: Hs02800695_m1; *B2M*: Hs00984230_m1). For each sample, the target and reference genes were assayed on the same card to exclude technical variations.

The relative threshold method (Crt method) was applied, which is a robust method that sets a threshold for each curve individually, based on the shape of the amplification curve, regardless of the height, or variability of the curve during its early baseline fluorescence. The expression of collagen genes across the samples was calculated using the equation ΔCrt, in which [ΔCrt = target gene Crt—the mean of reference genes Crt]. A lower cycle threshold value (Crt) indicates higher gene expression.

Statistical Analysis

All gene expression data (ΔCrt) are shown as the median with the interquartile range (IQR).

We verified the distribution of all continuous variables using the Shapiro–Wilk normality test to determine the appropriate tests for subsequent statistical comparisons. The expression data were not normally distributed. Therefore, the Mann–Whitney test was performed to compare the gene expression between the studied groups and clinical variables, such as gender, number of injuries (1 dislocation episode versus >1 dislocation episode), practice of physical activity involving the upper limbs and type of job (manual versus non-manual job). A χ^2 test was used to compare the gender distribution between cases and controls. Spearman's correlation was applied to evaluate the possible correlation between gene expression and age at surgery or duration of symptoms. A p-value of < 0.05 was considered statistically significant.

RESULTS

Patient Data and Clinical Outcomes

Table 1 shows the clinical outcomes of the shoulder instability patients. In our experimental group, 68.8% of patients with shoulder instability for less than one year (time between the onset and surgery) presented two or more dislocation episodes, whereas 92.3% of patients with shoulder instability for more than one year presented two or more dislocation episodes. The duration of the symptoms was associated with the number of dislocation episodes ($p = 0.008$, Mann–Whitney test).

Although no histological assessment was performed, a macroscopic evaluation during the arthroscopic procedure revealed that all shoulder instability patients presented a more flexible capsular aspect in the AI site.

Among the controls, 7 (87.5%) were males and 1 (12.5%) was female, and the median age at time of surgery was 31.44 years (IQR = 13.5). No significant difference was observed in the distribution of gender between the groups ($p = 1$). In addition, age at the time of surgery was not significantly different between the patients and controls ($p = 0.571$, Mann–Whitney test).

Differences Between the Cases and Controls

Table 2 shows the median and interquartile range of *TGFβ1*, *TGFβR1*, *LOX*, *PLOD1*, and *PLOD2* expression in the AI, AS, and P sites of the glenohumeral capsule in the patients and controls. The shoulder instability patients presented increased *PLOD2* expression in the AI portion of the capsule compared to the controls ($p = 0.020$). Moreover, *LOX* expression tended to be increased in the P portion of the capsule in the studied patients ($p = 0.058$). No other significant differences between the samples of cases and controls were detected ($p > 0.05$).

Gene Expression and Clinical Variables of Shoulder Instability Patients

LOX expression was increased [2.57 (1.39) vs 4.51 (0.40); $p = 0.048$] and *TGFβR1* was reduced [1.45 (0.43) vs 0.99 (0.01); $p = 0.048$] in the AI portion of the capsule of male compared to female patients. However, only two patients were female.

With regard to the number of injuries, *TGFβR1* expression was increased in the AI portion of the capsule in patients with recurrent shoulder dislocation compared to patients with a single episode of shoulder dislocation [1.37 (0.32) vs 1.70 (0.74); $p = 0.010$; Figure 1A]. In the P portion of the capsule, *PLOD2* was also upregulated in patients with recurrent shoulder dislocation [2.53 (0.58) vs 2.78 (0.45); $p = 0.049$; Figure 1B]. Conversely, *LOX* was increased in the P portion of the capsule of patients with a single shoulder dislocation episode [2.02 (0.703) vs 2.68 (1.54); $p = 0.025$; Figure 1C]. Furthermore, patients with only one dislocation episode presented increased expression of *LOX* in the P region of the capsule compared with controls [2.02 (0.703) vs 3.36 (1.44); $p = 0.007$]. The expression of *LOX* did not differ between patients with recurrent shoulder dislocations episodes and controls ($p > 0.05$, for all capsule regions).

In the AI region of the capsule, the expression of *LOX* ($\rho = 0.421$; $p = 0.029$; Figure 2A) and *TGFβR1* ($\rho = -0.479$, $p = 0.012$; Figure 2B) were correlated with the duration of symptoms. In the P region of the capsule, the expression of *PLOD2* ($\rho = -0.465$; $p = 0.025$; Figure 2C), *TGFβ1* ($\rho = -0.425$, $p = 0.043$; Figure 2D) and *TGFβR1* ($\rho = -0.446$, $p = 0.032$; Figure 2E) were correlated with the duration of symptoms.

Patients who engage in physical activity involving the upper limbs presented reduced *PLOD1* expression

Table 1. Distribution of the Clinical Outcomes of Shoulder Instability Patients

Variable	Distribution
Age at surgery, years [median (IQR)	28.5 (9)
Age of onset, years [median (IQR)	25 (9)
Gender [N (%)]	
Male	27 (93.1)
Female	2 (6.9)
Duration of condition, years [median (IQR)]	1 (2.1)
Duration of condition [N (%)]	
≤ 1 year	16 (55.2)
> 1 year	13 (44.8)
Number of injuries [N (%)]	
1 dislocation	6 (20.7)
> 1 dislocation	23 (79.3)
Physical activity involving the upper limbs [N (%)]	
No	12 (41.4)
Yes	17 (58.6)
Manual job [N (%)]	
No	20 (69)
Yes	9 (31)

N: number of patients. IQR: interquartile range.

in the AS portion of the capsule [1.31 (0.18) vs 1.06 (0.72); $p = 0.045$; Figure 3A]. In the P portion of the capsule, *PLOD2* expression was also reduced in patients who engage in physical activity involving the upper limbs compared to those that did not practice this type of physical activity [2.75 (0.74) vs 2.44 (0.38); $p = 0.033$; Figure 3B]. A tendency of reduced *TGFβ1* expression in the P portion of the capsule was also observed in these patients [1.02 (0.60) vs 0.76 (0.48); $p = 0.055$; Figure 3C].

No association between the expression of the studied genes and any other clinical variable was found in shoulder instability patients ($p > 0.05$).

DISCUSSION

The AI portion of the glenohumeral capsule of shoulder instability patients commonly exhibits macroscopic alteration,[32,33] such as the capsular deformation that was detected during surgical treatment in all the studied patients. In the present study, we found that *PLOD2* expression was increased in the AI portion of the capsule of patients compared with controls. In several connective tissues (for example, bone, tendon, ligaments, and cartilage)[24] and in fibrotic skin, the formation of collagen cross-links occur by the hydroxyallysine route.[24,34] In this pathway, a hydroxylysine residue in the collagen telopeptide (the terminal non-triple helical domain) is converted into aldehyde hydroxyallysine. Subsequently, the hydroxyallysine reacts with a hydroxylysine residue in the collagen triple helix to form functional cross-links. In contrast to PLOD1, PLOD2 is a lysyl hydroxylase that hydroxylates the telopeptides.[34] Therefore, the shoulder dislocation episode may lead to *PLOD2* up-regulation in an attempt to heal the capsule through the cross-linking of the new collagen fibrils by the hydroxyallysine route in the injured tissue.

Although we did not observe a significant difference between cases and controls in the P portion of the glenohumeral capsule, patients with more than two episodes of shoulder dislocation and with longer duration of symptoms presented increased expression of *PLOD2* in this capsule region. There is a reciprocal load-sharing relationship in the capsule, whereby tensile load in either the anterior or superior structures is simultaneously accompanied by laxity in the P or inferior portion, respectively.[33] The P region of the capsule of anterior shoulder instability patients also presents upregulation of *COL1A1*, *COL1A2*, *COL3A1*,[5] and other non-collagen ECM genes (unpublished data). Thus, our results reinforce the idea that the molecular alterations noted in the P region of the capsule may be

Table 2. Expression of Genes Involved in Collagen Cross-Linking and Its Regulation in the Glenohumeral Capsule of Patients With Shoulder Instability and Controls

	AI			AS			P		
Gene	Cases [ΔCrt; Median (IQR); $N = 29$]	Controls [ΔCrt; Median (IQR); $N = 8$]	p-value	Cases [ΔCrt; Median (IQR); $N = 29$]	Controls [ΔCrt; Median (IQR); $N = 8$]	p-value	Cases [ΔCrt; Median (IQR); $N = 29$]	Controls [ΔCrt; Median (IQR); $N = 8$]	p-value
LOX	2.64 (1.70)	3.37 (1.52)	0.285	2.65 (1.33)	3.18 (1.98)	0.104	2.55 (1.59)	3.36 (1.44)	0.058[b]
PLOD1	1.08 (0.61)	1.07 (0.47)	0.618	1.29 (0.40)	1.06 (0.72)	0.223	1.19 (0.52)	1.10 (0.42)	0.891
PLOD2	2.64 (0.85)	3.11 (1.08)	0.020[a]	2.66 (0.76)	2.61 (0.65)	0.968	2.59 (0.60)	2.85 (1.70)	0.089
TGFβ1	0.75 (0.58)	0.58 (0.52)	0.605	0.93 (0.49)	0.77 (0.51)	0.133	0.89 (0.48)	0.82 (1.71)	0.785
TGFβR1	1.42 (0.40)	1.47 (0.73)	0.883	1.51 (0.93)	1.61 (0.19)	0.968	1.68 (0.88)	2.08 (0.76)	0.219

AI: antero-inferior portion of the glenohumeral capsule; AS: antero-superior portion of the glenohumeral capsule; P: posterior portion of the glenohumeral capsule; IQR: interquartile range; N: number of samples. A lower delta cycle threshold value (ΔCrt) indicates higher gene expression. [a]Significant difference between groups by Mann–Whitney test ($p < 0.05$). [b]A tendency to increased expression in shoulder instability patients by Mann–Whitney test.

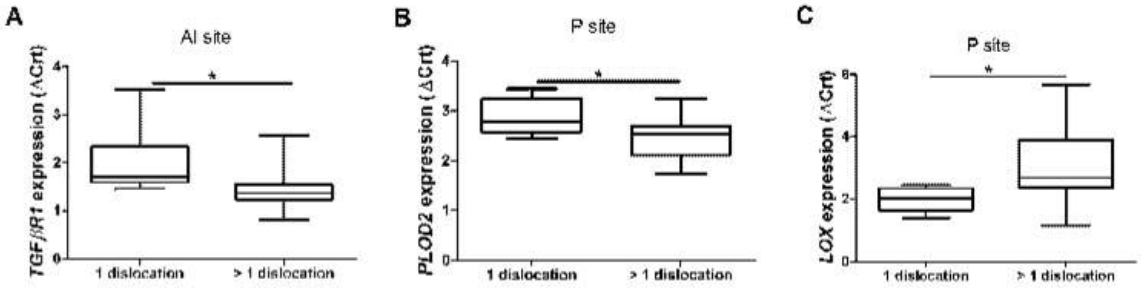

Figure 1. Gene expression by number of dislocation episodes. (A) Increased *TGFβR1* expression in the antero-inferior site of the glenohumeral capsule of shoulder instability patients with more than 1 dislocation episode compared to patients with 1 dislocation episode. (B) Increased *PLOD2* expression in the posterior site of the glenohumeral capsule of shoulder instability patients with more than 1 dislocation episode compared to patients with 1 dislocation episode. (C, B) Increased *LOX* expression in the posterior site of the glenohumeral capsule of shoulder instability patients with 1 dislocation episode compared to patients with more than 1 dislocation episode. 1 dislocation: gene expression in the capsule of patients who have experienced a single episode of shoulder dislocation ($N = 6$). > 1 dislocation: gene expression in the capsule of patients who have experienced more than one episode of shoulder dislocation ($N = 23$). A lower delta cycle threshold value (ΔCrt) indicates higher gene expression. $^*p$-value < 0.05 by Mann–Whitney test.

indicative of the biomechanic tissue disorders. Furthermore, the increased *PLOD2* expression in this region may be related to the continuation of the symptoms and their recurrence.

PLOD2, *TGFβ1*, and *TGFβR1* may also have a role in the disease etiology. We observed that the expression of these genes increases with the duration of disease in the P region. *PLOD2* seems to be regulated by *TGFβ1*.[17] Moreover, previous studies have shown that *TGFβR1* is a key element in the regulation of wound healing.[35] Therefore, the continuation of symptoms may contribute to activation of the TGFβ pathway in the P region.

On the other hand, the expression of *LOX* seems to be reduced with the disease. Although we observed that *LOX* expression tended to be increased in the P region of the patient's capsule, its expression was significantly higher in patients with only one dislocation episode compared to controls and compared to patients with recurrent dislocations. Moreover, this gene expression was inversely correlated to the duration of symptoms in the macroscopically injured capsule region. Thus, increased *LOX* expression seems to be occurring in an initial phase of the disease.

Our previous study demonstrated the increased expression of collagen fibrils genes across the capsule

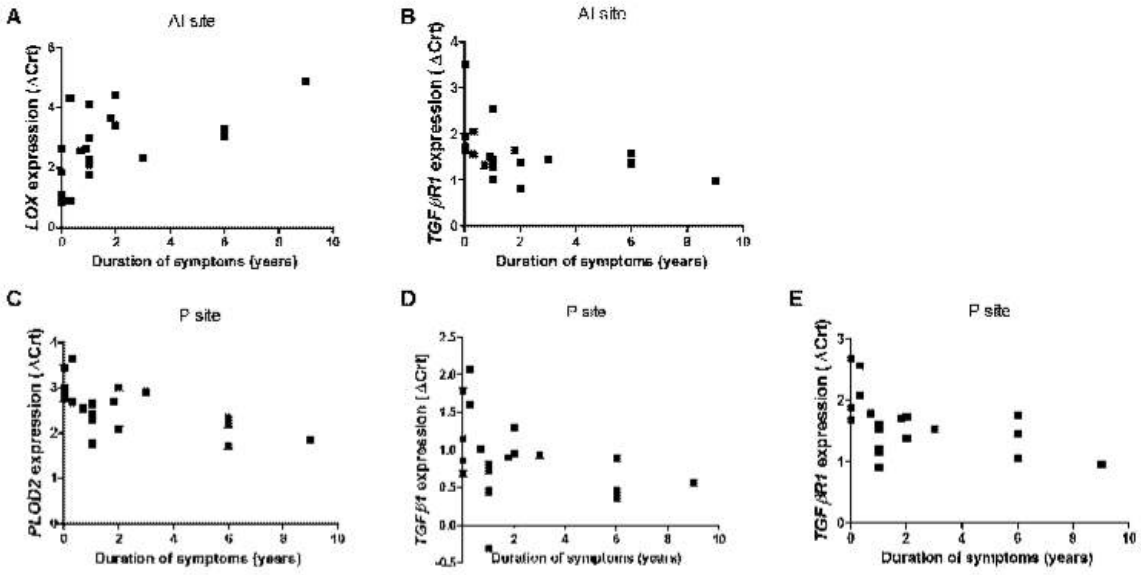

Figure 2. Correlation between gene expression and duration of shoulder instability symptoms (years). (A) *LOX* expression in the antero-inferior site of the glenohumeral capsule. (B) *TGFβR1* expression in the antero-inferior site of the glenohumeral capsule. (C) *PLOD2* expression in the posterior site of the glenohumeral capsule. (D) *TGFβ1* expression in the posterior site of the glenohumeral capsule. (E) *TGFβR1* expression in the posterior site of the glenohumeral capsule. A lower delta cycle threshold value (ΔCrt) indicates higher gene expression; 29 samples of each capsule site was used for each statistical analysis.

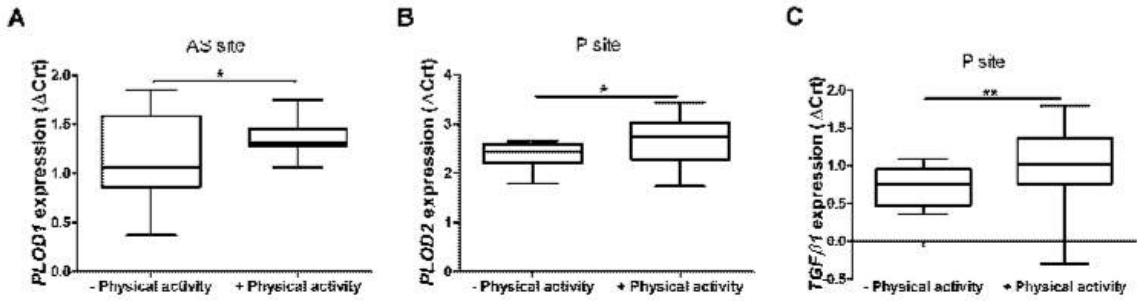

Figure 3. Gene expression by physical activity. (A) Reduced *PLOD1* expression in the antero-superior site of the glenohumeral capsule of shoulder instability patients that practiced physical activity compared to patients that did not practice physical activity. (B) Reduced *PLOD2* expression in the posterior site of the glenohumeral capsule of shoulder instability patients that practiced physical activity compared to patients that did not practice physical activity. (C) A tendency to reduced *TGFβ1* expression in the posterior site of the glenohumeral capsule of shoulder instability patients that practiced physical activity compared to patients that did not practice physical activity. Physical activity: gene expression in the capsule of patients that did not report the practice of physical activity involving the upper limbs ($N = 12$). +Physical activity: gene expression in the capsule of patients that reported the of practice physical activity involving the upper limbs ($N = 17$). A lower delta cycle threshold value (ΔCrt) indicates higher gene expression. $^{*}p$-value < 0.05 by Mann–Whitney. $^{**}p$-value $= 0.055$ by Mann–Whitney.

of shoulder instability patients compared to controls.[5] It is generally accepted that the total amount of enzymatic cross-linking is controlled by the expression of *LOX*. Several previous studies have demonstrated that collagen cross-link formation directly affects the strength of bones, tendons, and ligaments.[36,37] With concomitant collagen upregulation, we should expect *LOX* upregulation across the capsule. Therefore, the lack of *LOX* upregulation suggests that the new collagen fibrils may have reduced resistance to mechanical stress.

We observed that *PLOD2* and TGF*β1* were reduced in the P portion and *PLOD1* was reduced in the AS portion of the capsule of patients who undertook physical activity involving the upper limbs compared to those who did not. It is widely reported that physical activity involving the superior member can lead to biomechanical and structural capsule modifications, such as capsular tightness, especially in the P region.[38] Although additional investigations are still necessary, we hypothesize that these capsule modifications due to physical activity may explain the reduced *PLOD2*, *TGFβ1*, and *PLOD1* expression in the capsule of a subgroup of patients.

To our knowledge, this is the first study to evaluate *TGFβ1*, *TGFβR1*, *LOX*, *PLOD1*, and *PLOD2* gene expression in the glenohumeral capsule of shoulder instability patients. However, this study has some limitations. First, we evaluated a single time point (at the time of surgical repair); thus, we were unable to evaluate the dynamic regulation of gene expression. It is not possible to perform a longitudinal gene expression study in a human capsule sample. Therefore, to try to understand the modifications that occur with time, we performed the statistical correlation analyses between gene expression and duration of symptoms. Second, although no significant intra-articular pathology was detected in controls during the arthroscopic examination, it is important to highlight that the plastic deformation of the joint capsule cannot be easily detected through an arthroscopic or clinical exam. One other limitation concerns the tissue biopsy collection. The synovium is adhered to the capsule and cannot be separated from the capsule using arthroscopic instruments. The synovium may also contributes and influences the gene expression findings. Finally, some statistical analyses exhibited reduced power to detect significant differences between groups, and this was most likely due to the high degree of heterogeneity among patients with anterior shoulder instability. Therefore, false-negative results may have occurred.

In conclusion, we found increased *PLOD2* expression in the macroscopically injured region of the glenohumeral capsule of shoulder instability patients. Upregulation of *TGFβ1*, *TGFβR1*, and *PLOD2* seem to be related with the duration of symptoms, especially in the P region of the capsule. *LOX* upregulation seems to occur only in an initial phase of the disease. Therefore, *TGFβ1*, *TGFβR1*, *LOX*, and *PLOD2* may play a role in shoulder instability.

AUTHORS' CONTRIBUTIONS

Author Contributions Statement: PSB, MFL, BE, and MC conceived and designed the experiments. PSB and BE were responsible for sample collection. EAF, CC, CVA, and ACP were involved in clinical data collection. MFL was responsible for the genetic analysis. MFL and MCS were involved in statistical analysis. PSB and MFL were involved in literature search and wrote the first draft of the manuscript. All authors listed have contributed to all subsequent drafts, and have approved the final manuscript.

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1.9 Artigo IV - Belangero et al., 2016b

Objetivo: 1) comparar a expressão de genes que codificam proteínas da matriz extracelular (*COMP*, *FN1*, *TNC* e *TNXB*) da cápsula glenoumeral entre pacientes com IATO e controles; 2) investigar as associações entre a expressão desses genes e parâmetros clínicos da doença.

Casuística: Foram avaliadas amostras de três regiões da cápsula glenoumeral (região anteroinferior, anterossuperior e posterior) de 29 pacientes com IATO e 8 controles.

Principais resultados: A expressão de *COMP* estava reduzida (*errata submetida à revista*) e a expressão de *FN1 e TNC* estava aumentada (*errata submetida à revista*) na região cápsula anteroinferior dos casos investigados em relação aos controles. Adicionalmente, a expressão *TNC* estava aumentada na porção posterior da cápsula de pacientes. *COMP* também apresentou expressão reduzida na região anteroinferior em comparação à região posterior dos pacientes. Na região anteroinferior, *FN1* estava aumentado na cápsula de pacientes com mais de um ano de sintomas e com deslocamentos recorrentes em comparação com os controles. A expressão de *FN1* e *TNXB* estava correlacionada com a duração dos sintomas na região posterior.

Conclusão: A expressão de *COMP*, *FN1*, *TNC* e *TNXB* estava alterada por toda a cápsula de pacientes. O número de episódios de luxação pode modificar a expressão de *FN1*, *TNC* e de *TNXB* no tecido lesionado. A alteração na expressão de *COMP* pode estar associada com a integridade da cápsula após luxação do ombro, particularmente na porção macroscopicamente afetada.

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Differential expression of extracellular matrix genes in glenohumeral capsule of shoulder instability patients

Paulo Santoro Belangero[a*], Mariana Ferreira Leal[a,b*], Eduardo Antônio Figueiredo[b], Carina Cohen[a], Carlos Vicente Andreoli[a], Marília Cardoso Smith[b], Alberto de Castro Pochini[a], Benno Ejnisman[a], and Moises Cohen[a]

[a]Departamento de Ortopedia e Traumatologia, Universidade Federal de São Paulo, São Paulo, Brazil; [b]Departamento de Morfologia e Genética, Universidade Federal de São Paulo, São Paulo, Brazil

ABSTRACT

Anterior shoulder instability is a common orthopedic problem. After a traumatic shoulder dislocation, patients present a plastic deformation of the capsule. The shoulder instability biology remains poorly understood. We evaluated the expression of genes that encode the cartilage oligomeric matrix protein (*COMP*), fibronectin 1 (*FN1*), tenascin C (*TNC*) and tenascin XB (*TNXB*) in the glenohumeral capsule of anterior shoulder instability patients and controls. Moreover, we investigated the associations between gene expression and clinical parameters. The gene expression was evaluated by quantitative reverse transcription-polymerase chain reaction in the antero-inferior (macroscopically injured region), antero-superior and posterior regions of the capsule of 29 patients with shoulder instability and 8 controls. *COMP* expression was reduced and *FN1* and *TNC* expression was increased in the antero-inferior capsule region of cases compared to controls ($p < 0.05$). *TNC* expression was increased in the posterior capsule portion of shoulder instability patients ($p = 0.022$). *COMP* expression was reduced in the antero-inferior region compared to the posterior region of shoulder instability patients ($p = 0.007$). In the antero-inferior region, *FN1* expression was increased in the capsule of patients with more than one year of symptoms ($p = 0.003$) and with recurrent dislocations ($p = 0.004$) compared with controls. *FN1* and *TNXB* expression was correlated with the duration of symptoms in the posterior region ($p < 0.05$). Thus, *COMP, FN1, TNC* and *TNXB* expression was altered across the capsule of shoulder instability patients. Dislocation episodes modify *FN1, TNC* and *TNXB* expression in the injured tissue. *COMP* altered expression may be associated with capsule integrity after shoulder dislocation, particularly in the macroscopically injured portion.

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Introduction

Shoulder dislocation is observed in 1–2% of the population (1). Traumatic injuries account for 95% of shoulder dislocation episodes (2). Shoulder dislocations are common in young athletes who participate in competitive sports (3). After a dislocation episode, shoulder instability (SI) may occur with a recurrence rate of up to 100% in young athletes (4,5).

The anterior glenohumeral joint capsule is affected in about 90% of shoulder dislocations (6). SI patients present a plastic deformation of the capsule, which results in capsular laxity (7,8). Macroscopically, the antero-inferior (AI) region of the capsule is the most frequently injured site (8,9). Moreover, the deformation of AI region of the glenohumeral capsule is considered to be the main pathological consequence of shoulder dislocation (10).

Investigation of gene expression in human capsule samples may help in improving the understanding of SI. Furthermore, gene expression analysis will be important to guide patient management based on the molecular alterations detected in the injured tissue and also important for the development of new therapeutic options complementary to surgery. Previously, it was reported that the expression of collagen genes was increased in the AI, antero-superior (AS) and posterior (P) portions of the glenohumeral capsule in traumatic anterior SI patients compared to controls (11,12). Deregulated expression of genes involved in collagen cross-linking (such as *LOX* and *PLOD2*) was also reported in the capsule of SI patients (13). Moreover, increased *TGFβ1* and *TGFβR1* seem to be related with the maintenance of disease symptoms (13). Therefore,

CONTACT Mariana Ferreira Leal mariana.morf@epm.br Departamento de Ortopedia e Traumatologia, Universidade Federal de São Paulo, Rua Borges Lagoa, 783, CEP: 04038-031 São Paulo, SP, Brazil.
*Both authors contributed equally to this study.

these molecular alterations may have a role in the structure of collagen fibril and in the tissue healing process.

Although collagens are the most abundant proteins in the capsule, other non-collagen components of the extracellular matrix (ECM) may be important for the structure and function of the tissue. Cartilage oligomeric matrix protein (*COMP*) is a glycoprotein found in the ECM of joints that plays a catalytic role in fibrillogenesis (14). It has been suggested that COMP may be synthesized as a component of the healing response in many connective tissues (15). Fibronectin (FN) is a multidomain glycoprotein that plays a vital role in cell adhesion, tissue development, and wound healing (16). Increased expression of FN1 was previously described in rotator cuff tears and Achilles tendon injuries (17,18). Moreover, the tenascins (TN), including TNR, TNC and TNX, are a highly conserved family of large oligomeric glycoproteins found in the ECM of human tissues. TNR is expressed only in the brain, whereas TNC and TNX are expressed in several organs and tissues, including in the joints (19). TNC acts in several processes during the tissue injury, including inflammation, cell proliferation and migration, ECM synthesis and assembly, vascular remodeling and tissue remodeling [see review (20)]. Tenascin-C has a tightly regulated pattern of expression (21) and deregulated expression of TNC was previously reported in rotator cuff tears and impingement syndrome (22,23). It has been suggested that TNXB might regulate collagen synthesis or deposition (24).

We hypothesized that gene expression alterations may arise as a result of shoulder dislocation and may predispose to recurrent dislocation episodes. We therefore evaluated whether the *COMP*, *FN1*, *TNC* and *TNXB* expression in glenohumeral capsule differs (1) between SI patients and controls and/or (2) among capsule regions. Moreover, we (3) investigated the possible associations between gene expression and preoperative clinical parameters.

Methods

Patients

This is a case-control study (level 3 of evidence) approved by the ethics committee of the UNIFESP (approval number: CEP 51436; CAAE 01609812.9.0000.5505). All studied individuals signed an informed consent before data and sample collection.

We evaluated 29 outpatients with traumatic anterior shoulder dislocation from Hospital São Paulo of Universidade Federal de São Paulo (UNIFESP), Brazil. After the first episode of shoulder dislocation, patients were treated for at least two weeks with shoulder immobilization. All patients underwent arthroscopic surgical treatment for SI. The inclusion criteria were as follows: no history of previous surgery for an injured shoulder, positive apprehension test and a Bankart lesion on magnetic resonance imaging. The exclusion criteria were as follows: prior shoulder injection, presence of significant bone defects (humeral and/or glenoid greater than 20%), presence of clinical signs of posterior or multidirectional instability, presence of generalized joint hyperlaxity or hypermobility by Beighton score (25) and presence of associated lesions, such as superior lesion anterior posterior (SLAP) lesions detected during the surgery.

As control group, we included eight subjects who underwent arthroscopically assisted treatment for acromioclavicular dislocation. All controls were physically active. These individuals did not present any positive signs for this injury under anesthesia or history of SI. Moreover, we did not find any radiological indications of glenohumeral capsule alterations in controls. A standard complete joint examination with scope and probe during arthroscopy confirmed that the controls did not present any other concomitant pathology in the shoulder.

All patients answered a preoperative questionnaire that included questions regarding gender, age at surgery, age of onset, duration of symptoms, number of dislocation episodes and type of work. The preoperative clinical parameters of the SI patients are shown in table 1.

Tissue samples

During the arthroscopic procedure, tissue samples were obtained from AI, AS and P sites of the glenohumeral

Table 1. Distribution of the preoperative clinical parameters of shoulder instability patients.

Variable	Distribution
Age at surgery, years [median (IQR)]	28.5 (9)
Age of onset, years [median (IQR)]	25 (9)
Gender [N(%)]	
Male	27 (93.1)
Female	2 (6.9)
Duration of condition, years[a] [median (IQR)]	1 (2.1)
Duration of condition[a] [*N*(%)]	
≤ 1 year	16 (55.2)
> 1 year	13 (44.8)
Number of injuries [*N*(%)]	
1 dislocation	6 (20.7)
> 1 dislocation	23 (79.3)
Manual job [*N*(%)]	
No	20 (69)
Yes	9 (31)

[a]Duration between the first dislocation episode and the date of capsular biopsy. N: number of patients; IQR: interquartile range.

capsule of each patient. To minimize the variation of sampling, the tissue specimens were taken by two of the authors (PSB and BE).

The tissue samples were collected as previously described (11,13). The biopsy samples of AI and AS sites were obtained with the scope in the posterior portal and the basket grasper in the anterior portal. The AI specimen was taken from the most inferior region of glenohumeral capsule next to the inferior glenohumeral ligament. The AS specimen was taken in the direction of the anterior portal, below the biceps tendon, in the rotator interval area. The P specimen was taken in the direction of the posterior portal during the evaluation of the posterior capsulolabral complex with the scope in the anterior portal and the basket grasper in the posterior portal.

All tissue specimens were immediately immersed in RNAlater® solution (Qiagen, Germany) and then stored at −20 °C until RNA extraction.

RNA extraction

Total RNA was purified with an RNeasy mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Tissue Lyser LT equipment (Qiagen) was used in the mechanical lysis step. RNA concentration and quality were determined using a NanoDrop ND-1000 spectrophotometer (Thermo Scientifc, Wilmington, DE). RNA integrity was verified by 1% agarose gel electrophoresis. RNA samples were stored at −80 °C until further use.

mRNA expression analysis

Gene expression was evaluated by reverse-transcription quantitative polymerase chain reaction (RT-qPCR), which is a gold standard technique for the analysis of mRNA level (26). RT-qPCR gene expression quantifications were performed according to MIQE guidelines (27). Only RNA samples with the optical density (OD) 260/280 > 1.8 were used.

Gene expression was evaluated as previously described (13). Briefly, High-Capacity cDNA Archive kit (Life Technologies, Foster City, CA) was used for cDNA synthesis. qPCR were performed with 80-100 ng of cDNA input using TaqMan low-density array (TLDA) cards (Life Technologies) and ViiA 7 real-time PCR System (Life Technologies). Only inventoried TaqMan Gene Expression Assays (Life Technologies) were used for gene expression analysis.

The *HPRT1* and *B2M* genes were used as internal controls to normalize the sample input amount (28). All RT-qPCR reactions were performed in triplicate for all target genes (*COMP*: Hs00164359_m1; *FN1*: Hs00365052_m1; *TNC*: Hs01115665_m1; *TNXB*: Hs00372889_g1) and reference genes (*HPRT1*: Hs02800695_m1; *B2M*: Hs00984230_m1). To exclude technical variations, the target and reference genes were assayed on the same card.

We applied the cycle relative threshold method (Crt method), which is a robust method that sets a threshold for each curve individually based on the shape of the amplification curve, regardless of the height or variability of the curve during its early baseline fluorescence (29–32). The expression of target genes across the samples was calculated using the equation ΔCrt, in which [ΔCrt = target gene Crt − the mean of reference genes Crt]. A lower ΔCrt indicates higher gene expression.

Statistical analysis

All gene expression data (ΔCrt) are shown as the median with the interquartile range (IQR). The clinical parameters are shown as frequency (categorical variables) or median with IQR (continuous variables). We dichotomized the number of injuries (1 dislocation *versus* > 1 dislocation) since some patients were not able to specifically report the number of injury episodes.

A chi-square test was performed to compare the gender distribution between patients and controls. Shapiro-Wilk normality test was used to verify the distribution of all continuous variables and determine the appropriate tests for subsequent statistical comparisons. The expression data were not normally distributed. Therefore, the Mann–Whitney test was used to compare the gene expression between the studied groups and clinical variables. Wilcoxon Signed Rank test was used to compare the gene expression among the three studied regions of the capsule. Spearman's correlation was applied to investigate the possible correlation between gene expression and age at surgery, age of onset or duration of symptoms. The Spearman's Rho (correlation coefficient) below 0.40 represents a weak correlation, 0.40–0.59 a moderate correlation, 0.6–0.79 a strong correlation, and ≥0.80 a very strong correlation.

A p-value of ≤0.05 was considered statistically significant. Bonferroni adjustment of the p-value was applied for multiple comparisons, with the alpha level being divided by the number of comparisons (adjusted $\alpha = 0.05/3 = 0.0167$).

Results

Patient and clinical data

The duration of the symptoms was associated with the number of dislocation episodes ($p = 0.008$, Mann–Whitney test) in SI patients. Moreover, 68.8% of

patients with SI for less than one year (time between the onset of the condition and surgery) presented 2 or more dislocation episodes, whereas 92.3% of patients with SI for more than one year presented 2 or more dislocation episodes.

We observed that all SI patients presented a more flexible capsular aspect at the AI site in a macroscopic evaluation during the arthroscopic procedure.

Among the controls, the median age at time of surgery was 31.4 years (IQR=13.5) and seven (87.5%) were males and only one (12.5%) was female. The distribution of gender between the patients and controls was not significant different (p = 1, X^2 test). Additionally, the age at the time of surgery was not significantly different between the groups (p = 0.571, Mann–Whitney test).

Differences between the patients and controls

The SI patients presented reduced *COMP* expression in the AI portion of the capsule in relation to the controls (p = 0.022, Mann–Whitney test; Table 2). On the other hand, the expression of *FN1* was increased in the AI site (p = 0.007, Mann–Whitney test; Table 2). In addition, the SI patients presented higher *TNC* expression in the AI (p = 0.042, Mann–Whitney test; Table 2) and P (p = 0.022, Mann–Whitney test; Table 2) portions of the capsule compared to controls. *TNXB* expression was not significantly different between the patients and controls in the three portions of the shoulder capsule examined (p > 0.05, Mann–Whitney test; Table 2).

Differences between the capsule regions

COMP expression was reduced in the AI region in relation to P region of the glenohumeral capsule of SI patients (p = 0.007, Wilcoxon test followed by Bonferroni adjustment; Figure 1A). *COMP* expression was also reduced in the AI region compared to the AS region of the capsule of the patients (p = 0.023, Wilcoxon Signed Rank test). However, this finding did not reach statistical significance after Bonferroni adjustment (adjusted α = 0.05/3 = 0.0167). Although the difference was not statistically significant, *TNC* and *TNXB* were increased in the P region compared to the AI (p = 0.042; p = 0.042; respectively, Wilcoxon Signed Rank test) and AS (p = 0.023; p = 0.019; respectively, Wilcoxon Signed Rank test) regions in the capsules of the SI patients (Figure 1C and 1D).

Gene expression did not differ between the three capsule regions in the controls (p>0.05, Wilcoxon Signed Rank test).

Preoperative clinical parameters and gene expression

COMP expression in the AI portion seems to be reduced and *TNC* expression in the P portion seems to be increased with the number of dislocation. The tissue samples of patients with recurrent shoulder dislocation presented reduced *COMP* expression in the AI portion of the capsule [1.65 (2.98) *vs* –0.01 (2.04); p = 0.023, Mann–Whitney test; Figure 2A] and increased *TNC* expression in the P portion [–1.36 (2.04) *vs* –0.12 (1.65); p = 0.034, Mann–Whitney test; Figure 2B] in relation to controls. However, these observations were not statistical significant after Bonferroni adjustment.

On the other hand, the tissue samples of patients with recurrent shoulder dislocation presented a significant increased *FN1* expression in the AI portion in relation to controls [–5.65 (0.77) *vs* –4.85 (0.78); p = 0.004, Mann–Whitney test; Figure 2C]. Moreover, *FN1* expression tended to be increased in the P portion of the capsule in patients with recurrent shoulder dislocation compared to patients with a single episode of shoulder dislocation [–5.63 (1.17) *vs* –4.75 (0.75); p = 0.053, Mann–Whitney test; Figure 2D].

In the P region of the capsule, the expression of *FN1* (ρ=-0.535; p = 0.009, Spearman's correlation; Figure 3A) and *TNXB* (ρ = –0.572, p = 0.004, Spearman's correlation; Figure 3B) was moderately correlated with the duration of symptoms.

Table 2. Expression of non-collagen genes of extracellular matrix in the glenohumeral capsule of patients with shoulder instability and controls.

	AI			AS			P		
Gene	Cases [ΔCrt; Median (IQR)]	Controls [ΔCrt; Median (IQR)]	p-value	Cases [ΔCrt; Median (IQR)]	Controls [ΔCrt; Median (IQR)]	p-value	Cases [ΔCrt; Median (IQR)]	Controls [ΔCrt; Median (IQR)]	p-value
COMP	1.47 (2.72)	−0.01 (2.04)	0.022*	0.39 (3.68)	1.59 (5.63)	0.417	0.32 (3.80)	0.11 (3.36)	0.380
FN1	−5.64 (0.71)	−4.85 (0.78)	0.007*	−5.63 (0.84)	−4.91 (1.33)	0.223	−5.38 (1.11)	−4.82 (3.57)	0.266
TNC	−0.66 (1.52)	0.36 (1.31)	0.042*	−0.75 (0.92)	0.61 (1.09)	0.123	−1.31 (1.98)	−0.12 (1.65)	0.022*
TNXB	−2.67 (1.13)	−2.79 (1.03)	0.330	−2.52 (1.26)	−2.86 (2.12)	0.903	−2.93 (0.86)	−2.68 (1.78)	0.545

*Significant difference between groups by Mann–Whitney test (p < 0.05). AI: antero-inferior portion of the glenohumeral capsule; AS: antero-superior portion of the glenohumeral capsule; P: posterior portion of the glenohumeral capsule; IQR: interquartile range. A lower delta cycle threshold value (ΔCrt) indicates higher gene expression.

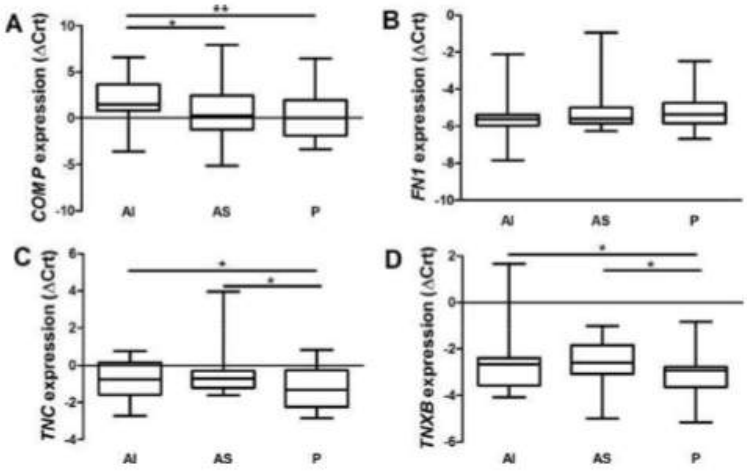

Figure 1. Expression of non-collagen genes of extracellular matrix in the glenohumeral capsule of patients with shoulder instability. (A) *COMP* expression; (B) *FN1* expression; (C) *TNC* expression; (D) *TNXB* expression. A lower delta cycle threshold value (ΔCrt) indicates higher gene expression. AI: antero-inferior portion of the capsule; AS: antero-superior portion of the capsule; P: posterior portion of the capsule. *0.0167 < *p*-value < 0.05 by Wilcoxon test; **p-value < 0.0167 by Wilcoxon test followed by Bonferroni adjustment.

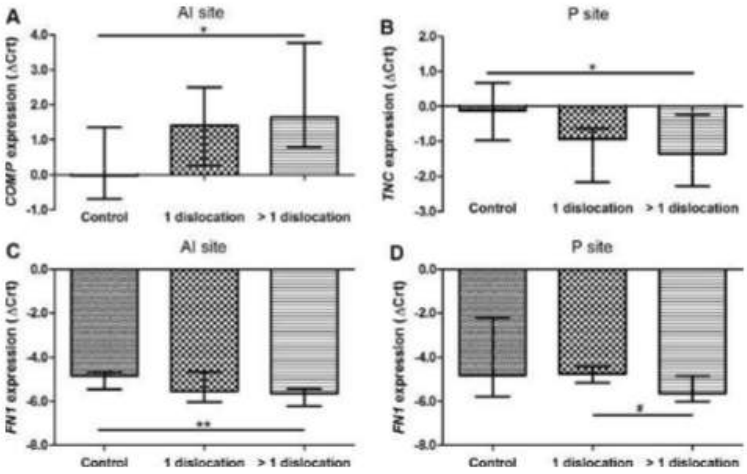

Figure 2. Expression of non-collagen genes of extracellular matrix by number of dislocation episodes. (A) *COMP* expression in antero-inferior site; (B) *TNC* expression in posterior site; (C) *FN1* expression in antero-inferior site; (D) *FN1* expression in posterior site. A lower delta cycle threshold value (ΔCrt) indicates higher gene expression. AI: antero-inferior portion of the capsule; P: posterior portion of the capsule. * 0.0167 < *p*-value < 0.05 by Mann–Whitney test. **p-value < 0.0167 by Mann–Whitney test followed by Bonferroni adjustment; $^{\#}p = 0.053$.

Additionally, reduced *COMP* [2.13 (2.45) *vs* −0.01 (2.04); $p = 0.03$, Mann–Whitney test; Figure 4A] expression and increased *FN1* [−5.93 (0.77) *vs* −4.85 (0.78); =0.003, Mann–Whitney test followed by Bonferroni adjustment; Figure 4B] and *TNC* [−0.82 (1.27) *vs* 0.36 (1.31); $p = 0.02$, Mann–Whitney test; Figure 4A] expression were observed in the AI portion of the capsule of patients with more than 1 year of symptoms in relation to controls. However, only *FN1* was significantly altered in SI patients after Bonferroni correction.

Although the difference was not statistically significant after Bonferroni adjustment, patients with less than 1 year of SI symptoms also presented reduced *COMP* [1.18 (2.95) *vs* −0.01 (2.04); $p = 0.05$, Mann–Whitney test; Figure 4A] and increased *FN1* [−5.62 (0.66) *vs* −4.85 (0.78); $p = 0.05$, Mann–Whitney test; Figure 4B] expression in the AI site and increased *TNC* [−1.68 (1.89) *vs* −0.12 (1.65); $p = 0.021$, Mann–Whitney test; Figure 4A] expression in the P portion of the capsule in relation to controls.

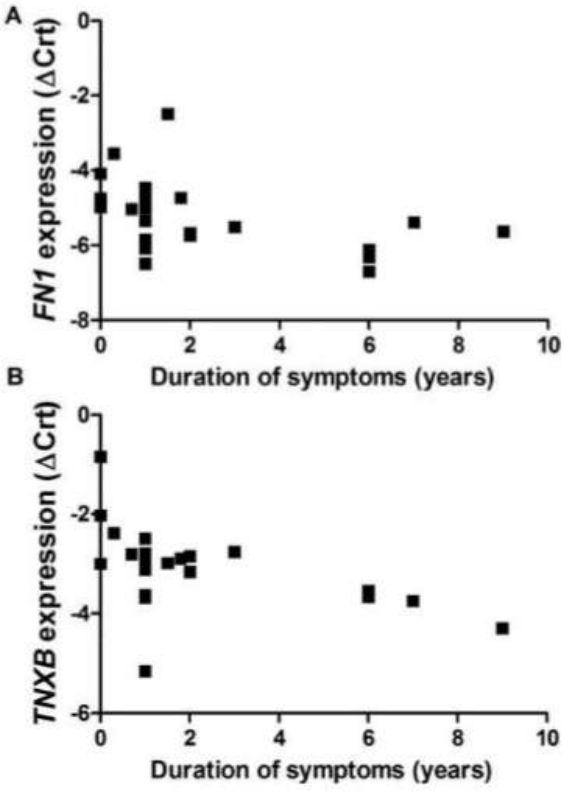

Figure 3. Correlation of *FN1* (A) and *TNXB* (B) expression and duration of shoulder instability symptoms (years) in the posterior site of the glenohumeral capsule. A lower delta cycle threshold value (ΔCrt) indicates higher gene expression.

No association or correlation between gene expression or any other clinical variable was found in SI patients ($p > 0.05$, Mann–Whitney test or Spearman's correlation).

Discussion

Proteoglycans and glycoproteins maintain the homeostasis of the ECM of the joints by regulating collagen fibril assembly (17). The AI portion of the glenohumeral capsule of SI patients commonly exhibits macroscopic alterations (7,8), such as the capsular deformation that was detected during surgical treatment in all the studied patients. Here, we found that the expression of *COMP, FN1* and *TNC* was altered in the AI region of the capsule of SI patients. Our results suggest that these genes may have a role in SI.

TNC is usually expressed during tissue repair and in pathological situations, such as chronic inflammation. TNC interacts with several other ECM molecules and cell-surface receptors, thus affecting tissue architecture, tissue resilience and cell responses. TNC induces pro-inflammatory cytokines and modulate cell migration, proliferation and cellular signaling (20). Deregulated expression of TNC was previously reported in rotator cuff tear and in impingement syndrome (22,23). In the impingement syndrome, TNC seems to be an essential aspect of the pathology at all stages (23).

Here, we detected increased *TNC* mRNA expression in a macroscopic and in a non-macroscopic injured region (P portion) of the capsule. Moreover, *TNC*

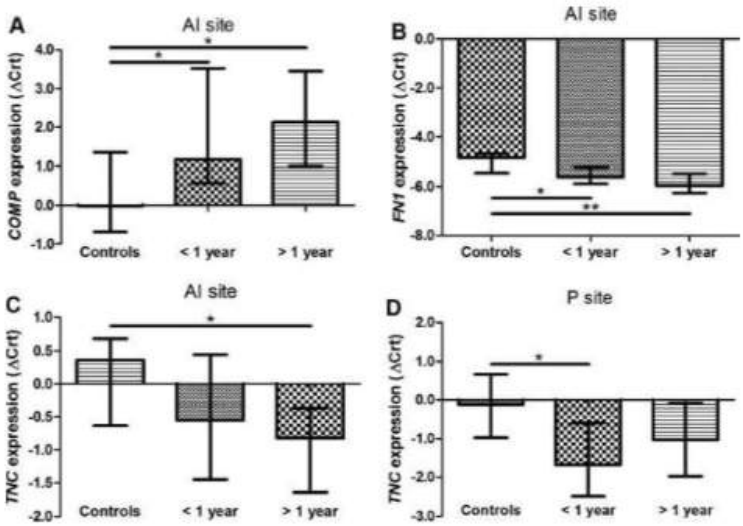

Figure 4. Expression of non-collagen genes of extracellular matrix by duration of symptoms. (A) *COMP* expression in antero-inferior site; (B) *FN1* expression in antero-inferior site; (C) *TNC* expression in antero-inferior site; (D) *TNC* expression in posterior site. AI: antero-inferior portion of the capsule; P: posterior portion of the capsule. *0.0167 < *p*-value < 0.05 by Mann–Whitney test. **p-value < 0.0167 by Mann–Whitney test followed by Bonferroni adjustment.

expression tended to be increased in the capsule P portion compared to the AI and AS regions in the studied patients. Previously, a macroscopic analysis of the collagen fiber bundle architecture in the AI region of the glenohumeral capsule revealed that a system of bundles that form a spiral arrangement allows the entire capsule to resist tensile and shear loads (33). Therefore, there is a reciprocal load-sharing relationship in the capsule, whereby the tensile load in either the anterior or superior structures is simultaneously accompanied by laxity in the P or inferior portion, respectively (8). Our investigation demonstrated that *TNC* alteration may be a key molecular event in SI and that anterior shoulder dislocation might lead to molecular alterations across the capsule even in patients without multidirectional instability, which is in agreement with previous molecular studies of our group (11,13).

FN1 was also increased in the macroscopic injured portion of the capsule in the SI patients, especially in patients with recurrent dislocation episodes and with more than one year of symptoms. Increased FN1 expression was previously described in tendon injuries, such as rotator cuff tears and Achilles tendon injuries (17,18,22). In a mouse model of flexor digitorum longus tendonplasty, it was demonstrated that *Fn* was upregulated during the proliferative phase of tendon (34). In an equine model, Fn was detected in the tendon within one week after injury, and it was still detectable in the healing tendon at one month after injury; however, two months later, it was no longer detectable (35). This preliminary expression of Fn is believed to affect adhesion formation, possibly through the roles of Fn in fibrin cross-linking, fibroblast chemotaxis and cell adhesion to the substratum during tendon healing (36,37). Therefore, increased *FN1* expression may also be a marker of early capsule injury.

Although we did not observed a significant difference between the patients and controls in the capsule P portion, patients with more the 2 episodes of shoulder dislocation and with a longer duration of symptoms presented increased expression of *FN1* in this capsule region. Thus, our results suggest that *FN1* expression in the capsule P portion may be also related with the maintenance of disease symptoms and therefore the SI.

We were not able to detect a significant difference between the patients and controls concerning *TNXB* expression; however, we cannot exclude the possibility that this gene may play a role in SI. As for *FN1*, we observed that *TNXB* expression increased with the duration of symptoms in the capsule P portion, and thus, its alteration may also be a result of capsule modifications that occur after a dislocation episode. Moreover, *TNXB* expression was increased in the capsule P portion compared to the AI and AS portions of the patients. TNXB seems to regulate collagen synthesis or deposition (24), and therefore may have a role in capsule injury and healing process.

COMP alteration appeared to be more restricted to the macroscopic tissue; the expression of this gene was reduced in the AI portion of the capsule in patients compared to controls. *COMP* expression also differed in the AI portion compared to the paired AS and P portions of the capsule in SI patients, and these non-macroscopic injured regions also present other molecular alterations, such as increased expression of *TNC* and collagen genes (11). Additionally, *COMP* reduced expression was mainly observed in the AI region of the capsule of patients with recurrent shoulder dislocation episodes and more than one year of symptoms. Previous studies in equines demonstrated that Comp may have a role in tendon functionality, and its levels have been shown to correlate with tendon strength at skeletal maturity (38). Comp is a highly labile matrix constituent that is more readily shed from skeletally mature tendons as a consequence of aging, exercise and injury (38,39), and it may be an indicator of ECM integrity (39). It was proposed that the identification of low Comp levels in tendon during growth would indicate horses prone to tendon injury (38). Our results suggest that *COMP* reduced expression may also be associated with the lack of capsule integrity after shoulder dislocation. Although the expression of other collagen and non-collagen genes was altered to favor the healing process (11, 13), the *COMP* down-regulation in the AI region of the capsule indicated that this process was not complete. This finding may contribute to the elucidation of the molecular mechanisms that lead to the high recurrence rate of anterior dislocations after the first episode of shoulder dislocation. Moreover, if confirmed in a larger sample set, *COMP* expression may be a parameter of glenohumeral capsule integrity.

To our knowledge, we originally evaluated *COMP, FN1, TNC* and *TNXB* gene expression in SI patients and controls. We believe that the increasing knowledge of the role of the selected genes adds to the understanding of the disease physiopathology. Taking together, our findings and those previously published show that clinical evaluation can overlook that important molecular alterations involved in structural tissue injury occur in the glenohumeral capsule even after a single episode of dislocation. It is important to underline that this study did not aimed to suggest new treatment or diagnostic tools. However, the better understanding of the molecular aspects is a key step

in dealing with any disease. Future studies targeting the evaluation of the mechanisms involved in the studied genes expression regulation and their protein product are still necessary. Moreover, further studies targeting other genes are crucial to decipher the complex mechanism of the tissue injury or regeneration.

Thus, this study has some limitations. First, the protein product analysis of these genes is still necessary since the protein function is also affected by posttranscriptional and posttranslational regulation. To avoid additional damages in the capsule, we obtained just a small tissue sample of each region during surgery. Therefore, we were not able to perform protein analyses and histological evaluation. Concerning the tissue biopsy collection, it is important to highlight that the synovium is adhered to the capsule and cannot be separated from the capsule using arthroscopic instruments. The synovium may also contribute to the gene expression findings. Finally, false-negative results may have occurred since some statistical analyses exhibited a reduced power to detect significant differences between the groups, and this was probably due to the high degree of heterogeneity among the patients with traumatic anterior SI.

In conclusion, we observed the altered *COMP, FN1, TNC* and *TNXB* expression across the capsule of shoulder dislocations patients. An imbalance in the expression ratio of these genes, particularly in the AI and P sites, was detected. These molecular alterations may lead to modifications of the tissue structure and the healing process. The *FN1, TNC* and *TNXB* altered expression suggest an attempt to tissue healing in the capsule. Conversely, *COMP* reduced expression may be associated with the lack of capsule integrity after shoulder dislocation, particularly in the macroscopically injured portion.

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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LIMITAÇÕES DO ESTUDO

LIMITAÇÕES DO ESTUDO

Abaixo, enumeramos as principais limitações do presente estudo:

1) O número de amostras avaliadas, especialmente de indivíduos controle, foi pequeno e, com isso, algumas análises estatísticas apresentaram baixo poder. Dessa forma, os nossos achados deveriam ser replicados em outras coortes.

2) A cápsula da AGU apresenta uma localização topográfica muito próxima ao tecido sinovial e, portanto, é difícil garantir uma coleta de material biológico, exclusivamente da cápsula, apenas sob visão macroscópica, uma vez que essas duas estruturas estão intimamente ligadas. O tecido sinovial pode também ter contribuído para os presentes achados.

3) Por apresentar um desenho de corte transversal, o presente estudo não nos permite compreender se o perfil de expressão gênica encontrado é causa ou efeito da doença. Contudo, mesmo sem encontrar essa resposta, podemos inferir que os genes diferencialmente expressos no tecido lesionado apontam o seu envolvimento e a sua relevância para a fisiopatologia da doença.

CONCLUSÕES

CONCLUSÕES

1.10 Gerais

- Identificamos alterações na expressão de genes codificadores de colágenos, genes envolvidos na modulação a síntese de fibras de colágenos ou que estão envolvidos no *crosslink* de colágenos, e de outros genes não-colágenos da matriz extracelular na cápsula glenoumeral de pacientes com instabilidade do ombro;
- Encontramos genes que podem ser relevantes para a fisiopatologia e/ou para a evolução da instabilidade anterior traumática do ombro, uma vez que observamos alteração na expressão em pacientes quando comparados com controles;
- Apesar dos pacientes possuírem IATO, as alterações na expressão desses genes foram detectadas em todas as regiões da cápsula avaliada, incluindo regiões sem alterações macroscópicas;
- Encontramos um número maior de genes envolvidos na região AI (região macroscopicamente afetada) em relação às outras regiões estudadas.

1.11 Comparação intergrupos (pacientes *vs* controles)

- A expressão de *COL1A1*, *COL3A1*, *PLOD2*, *FN1* e *TNC* estava aumentada e a expressão de *COMP* estava reduzida na região AI (macroscopicamente afetada) da cápsula de pacientes com IATO;
- A expressão de *COL1A1*, *COL1A2*, *COL3A1* estava aumentada na região anterossuperior e a expressão de *COL1A1*, *COL1A2*, *COL3A1* e *LOX* estava aumentada na região P da cápsula de pacientes com IATO, mostrando que as demais regiões da cápsula também podem ser afetadas nessa doença;
- Os genes supramencionados podem ser relevantes para a fisiopatologia da instabilidade traumática do ombro.

1.12 Comparação intragrupo de pacientes

1.12.1 Comparação entre regiões

- O único gene que evidenciou alteração da sua expressão na comparação entre as regiões estudadas foi o *COL5A1*, e essa alteração foi vista apenas na região AI em relação às outras regiões da cápsula glenoumeral de pacientes com IATO. Esse achado molecular pode ser crítico para que essa região evidencie alteração

também ao nível morfológico macroscópico.

1.12.2 Tempo de doença

- A expressão de *COL1A1*, *LOX* e *COMP* parece reduzir e a expressão de *TGFB1, FN1* e *TNC* parece aumentar quanto maior o tempo de sintomas da doença na região AI da cápsula glenoumeral de pacientes com IATO. Em adição, a expressão de *PLOD2*, *TGFB1*, *TGFBR1* e *TNXB* parece aumentar quanto maior o tempo de sintomas da doença na região P. Assim, a modulação da expressão desses genes pode estar relacionada à gravidade da doença.

1.12.3 Número de episódios

- A expressão de *TGFBR1* e *FN1* estava aumentada na região AI e P de pacientes com episódios recorrentes de luxação. A expressão de *TNC* também estava aumentada na região P de pacientes com episódios recorrentes de luxação. Assim, essas alterações podem estar relacionadas com a gravidade da doença;
- A expressão de *COMP* estava reduzida na região AI de pacientes com episódios recorrentes de luxação. Esse achado ressalta que a baixa expressão de COMP pode estar envolvida relacionada a falta de integridade da cápsula, especialmente após recorrentes episódios de luxação;
- A expressão de *COL1A1*, *COL3A1* e *COL5A* estava aumentada na região AI e a expressão de *LOX* estava aumentada na região P de pacientes com um único episódio de deslocamento. Esses resultados ressaltam que um único episódio de deslocamento pode levar a alterações na expressão de genes importante para a estrutura da cápsula, podendo estar relacionado a uma tentativa de reparo desse tecido.

ANEXOS

ANEXOS

1.13 Anexo 1: Aprovação do Comitê de Ética em Pesquisa UNIFESP/HSP N°1085/11

São Paulo, 27 de outubro de 2011
CEP Nº: **1085/11**

Ilmo(a) Sr(a
Pesquisador(a):PAULO SANTORO BELANGERO
Disciplina/Departamento: Traumatologia
Pesquisadores associados: Sintia Iole Nogueira Belangero, Moises Cohen (orientador)

Parecer Consubstanciado do Comitê de Ética em Pesquisa da Universidade Federal de São Paulo/Hospital São Paulo

TÍTULO DO ESTUDO: Estudo de expressão gênica: identificação de genes relevantes no desenvolvimento da instabilidade anterior do ombro .

CARACTERÍSTICA PRINCIPAL DO ESTUDO: Intervenção diagnóstica

RISCOS ADICIONAIS PARA O PACIENTE: Risco mínimo, envolvendo coleta de sangue e biópsia de tecido

OBJETIVO DO ESTUDO: Avaliar pacientes com instabilidade traumática anterior do ombro quanto ao padrão da expressão dos genes codificadores do colágeno tipo I (COL1A1, COL1A2), tipo III (COL3A1) e tipo IV (COL5A1), comparando: a) diferentes regiões da cápsula articular do ombro (acometidas e não acometidas); b) cápsula (tecido alvo) e sangue (tecido periférico).

RESUMO: Serão avaliados 30 pacientes com instabilidade traumática anterior do ombro, recrutados no serviço de Ortopedia e Traumatologia do Hospital São Paulo, incluindo pacientes provenientes do Pronto-Socorro e dos Ambulatórios. Serão incluídos pacientes adultos, de ambos os sexos, com o diagnóstico de instabilidade traumática anterior do ombro cujo tratamento indicado seja a cirurgia artroscópica. O diagnóstico será realizado com base na história clínica, no exame físico (teste da apreensão positivo, no qual reproduzimos a posição de risco para luxação e o paciente apresenta apreensão) e em exames subsidiários. Será realizada avaliação clínica, avaliação laboratorial em sangue periférico e em biópsias de cápsula das regiões ântero-inferior, ântero superior e posterior. Essas biópsias serão colocadas em tubos contendo uma 2mL de RNAlater (Qiagen, Alemanha), um reagente estabilizador de RNA e posteriormente, armazenados em freezer com temperatura de -80oC. Será realizado estudo da expressão gênica. Os genes encontrados que se apresentarem diferencialmente expressos entre os grupos serão analisados quanto ao padrão de metilação das ilhas CpG e quanto à presença de polimorfismos funcionais. Será realizado um estudo prospectivo utilizando como controle uma região do tecido alvo não afetada pela lesão, analisando-se as seguintes variáveis: independentes: regiões da cápsula e sangue periférico; dependentes: quantificação da expressão dos genes.

MATERIAL E MÉTODO: Estão descritos os procedimentos do estudo. RECOMENDAÇÃO DO CEP: O CEP solicita ao pesquisador que, nos relatórios parciais que serão encaminhados ao CEP daqui 12 meses, comunique também o consentimento dos pacientes quanto ao armazenamento ou não de materiais para pesquisa futuras e quanto sua vontade em ser informado sobre essas pesquisas

TCLE: Apresentado adequadamente, conforme solicitação do CEP-Unifesp

DETALHAMENTO FINANCEIRO: FAPESP - R$ 22905,00

CRONOGRAMA DO ESTUDO: 18 meses

PRIMEIROS RELATÓRIOS PARCIAIS PREVISTOS PARA : 21/10/2012 e 16/10/2013

O Comitê de Ética em Pesquisa da Universidade Federal de São Paulo/Hospital São Paulo ANALISOU e APROVOU o projeto de pesquisa referenciado.
1. Comunicar toda e qualquer alteração do projeto e termo de consentimento livre e esclarecido. Nestas circunstâncias a inclusão de pacientes deve ser temporariamente interrompida até a resposta do Comitê, após análise das mudanças propostas.
2. Comunicar imediatamente ao Comitê qualquer evento adverso ocorrido durante o desenvolvimento do estudo.
3. Os dados individuais de todas as etapas da pesquisa devem ser mantidos em local seguro por 5 anos para possível auditoria dos órgãos competentes.

Atenciosamente,

Prof. Dr. José Osmar Medina Pestana
Coordenador do Comitê de Ética em Pesquisa da
Universidade Federal de São Paulo/Hospital São Paulo

1.14 Anexo 2: Aprovação do Comitê de Ética em Pesquisa UNIFESP/HSP CAAE Nº 01609812.9.0000.5505

Plataforma Brasil - Ministério da Saúde

Universidade Federal de São Paulo - UNIFESP/ Hospital São Paulo

PROJETO DE PESQUISA

Título: AFECÇÕES ORTOPÉDICAS RELACIONADAS À PRÁTICA ESPORTIVA: ASPECTOS CLÍNICOS, GENÉTICOS E MOLECULARES

Área Temática:

Pesquisador: Mariana Ferreira Leal **Versão:** 2

Instituição: Universidade Federal de São Paulo - UNIFESP/EPM **CAAE:** 01609812.9.0000.5505

PARECER CONSUBSTANCIADO DO CEP

Número do Parecer: 51436

Data da Relatoria: 29/06/2012

Apresentação do Projeto:

De acordo com o parecer consubstanciado do CEP 19607 de 09/05/2012.

Objetivo da Pesquisa:

De acordo com o parecer consubstanciado do CEP 19607 de 09/05/2012.

Avaliação dos Riscos e Benefícios:

De acordo com o parecer consubstanciado do CEP 19607 de 09/05/2012.

Comentários e Considerações sobre a Pesquisa:

De acordo com o parecer consubstanciado do CEP 19607 de 09/05/2012.

Considerações sobre os Termos de apresentação obrigatória:

nada consta

Recomendações:

nada consta

Conclusões ou Pendências e Lista de Inadequações:

Pendências atendidas

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

As pendências foram atendidas, o colegiado acatou o parecer do relator. Projeto aprovado.

SAO PAULO, 06 de Julho de 2012

Assinado por:
José Osmar Medina Pestana

1.15 Anexo 3: Prêmio do *International Society of Arthroscopy, Knee Surgery and Orthopaedic Sports Medicine* (ISAKOS)

2013 ISAKOS Congress Awards

Richard B. Caspari Award

Award Recipient: Paulo Santoro Belangero, MD

Abstract/Presentation Title: Expression Profile of Collagen Genes in Shoulder Instability

Ranking: First Place

Sponsored By: DePuy Mitek

Presenter(s): G. Arce, G. Bain, E. Itoi, Tom Borg, David Hook

ISAKOS Congress - Toronto 2013
Abstract Preview

Below is a preview of your abstract.

Expression Profile of Collagen Genes in Shoulder Instability

Paulo Santoro Belangero , MD , Sao Paulo , SP , BRAZIL
Mariana Ferreira Leal , PhD , Sao Paulo , Please select , BRAZIL
Gabriel Esquitini Machado , Campinas , , BRAZIL
Eduardo Antonio De Figueiredo , MD , São Paulo , SP , BRAZIL
Carlos Vicente Andreoli , Sao Paulo , SP , BRAZIL
Carina Cohen , MD , São Paulo , , BRAZIL
Bernardo Barcellos Terra , MD , Vitoria , ES , BRAZIL
Gustavo Cara Monteiro , MD , São Paulo , SP , BRAZIL
Alberto Castro Pochini , MD , São Paulo , SP , BRAZIL
Sintia Iole Nogueira Belangero , PhD , Sao Paulo , , BRAZIL
Benno Ejnisman , MD , São Paulo , SP , BRAZIL
Moises Cohen , MD, PhD , São Paulo , SP , BRAZIL

Federal University of Sao Paulo, Sao Paulo, Sao Paulo, BRAZIL

Summary: Collagens gene expression was compared among three sites of shoulder capsule of patients with shoulder instability. COL5A1 expression was reduced in antero-inferior compared to the antero-superior portion.

Introduction:
Shoulder instability is a common affection, especially in young male athletes.
In addition of a traumatic event, it has been demonstrated that a genetic component is involved in the etiology of shoulde instability. The investigation of genes differentially regulated in shoulder instability may help the improvement of prognos determination and patient management.

Methods:
We have compared COL1A1, COL1A2, COL3A1 and COL5A1 gene expression among antero-inferior, antero-superior a posterior portions of shoulder capsule of patients with the diagnosis of traumatic anterior shoulder instability. Tissue sam were obtained from three different sites of
glenohumeral capsule of 20 patients with shoulder instability. In addition, one fragment of the antero-superior shoulder ca of a patient with acromioclavicular injury was obtained. Samples were collected during arthroscopic surgery. Tissue spec were immediately immersed in RNAlater solution and then stored at -20 oC until RNA extraction. Collagens gene expres were evaluated by quantitative reverse transcription polymerase chain reaction with primers and TaqMan probes. ACTB GAPDH genes were also evaluated as endogenous control genes. Relative quantification of gene expression was meas using delta-delta Ct method and the tissue sample of a patient with acromioclavicular injury as a calibrator. Expression d were z-score transformed and the outliers were excluded. Analyses of each gene expression were performed by paired s T-test with Bonferroni adjustment for multiple comparisons.

Results:
In the present study, COL1A1, COL1A2 and COL3A1 expression did not differ among the three portions of shoulder cap (p>0,016). However, COL5A1 expression was significantly reduced in antero-inferior portion compared to antero-superio region of glenohumeral capsule in patients with
shoulder instability (p=0.012).

Discussion and Conclusion:
The COL5A1 gene is related to the structural form of type I collagen (which isthe most frequent type in the capsule tissue this pattern of expression may be related to a higher degree of laxity in the inferior capsule that could predisposes anteri shoulder instability. This findings may represent a pathologic change in the intrinsic characteristics of the shoulder capsu patients with shoulder instability.

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APÊNDICES

APÊNDICES

Nessa sessão, serão apresentados, os artigos publicados durante o período do doutorado em que o aluno é coautor.

1.16 Apêndice 1: de Castro Pochini et al., 2014

Clinical Considerations for the Surgical Treatment of Pectoralis Major Muscle Ruptures Based on 60 Cases

A Prospective Study and Literature Review

Alberto de Castro Pochini,*† PhD, Carlos Vicente Andreoli,† PhD, Paulo Santoro Belangero,† MD, Eduardo Antonio Figueiredo,† MD, Bernardo Barcellos Terra,† MD, Carina Cohen,† MD, Marilia dos Santos Andrade,‡ PhD, Moises Cohen,§ PhD, and Benno Ejnisman,‖ PhD
Investigation performed at the Sports and Traumatology Center, Federal University of São Paulo, São Paulo, Brazil

Background: Early recognition of the clinical signs of ruptures of the pectoralis major muscle (PMM) in athletes by orthopaedic surgeons, physical therapists, and physical trainers may prove to be critical for patient access to surgical treatment while the injury is still in the acute phase.

Hypothesis: Total ruptures of the PMM may yield a better outcome with surgical treatment than with nonoperative treatment in athletes.

Study Design: Cohort study; Level of evidence, 2.

Methods: A prospective study was performed on 60 patients with total ruptures of the PMM. The patients were followed from 1997 to 2012, with a physical examination every 6 months for the first 2 years and every 12 months thereafter. The patients' mean age was 31.21 years, and the mean length of follow-up was 48.25 months. The surgical treatment methods included reinsertion of the tendon in 51% of the patients and nonoperative treatment in 49% of the patients. All of the patients were evaluated using the Bak criteria.

Results: The bench-press exercise was associated with 80% of the PMM ruptures (48 patients). Forty-one of the patients with chronic ruptures were seen after 3 months (80%). The outcomes were poor in 9 patients from the nonoperative group (31%) and in 3 patients from the surgical group (9.7%); the outcomes were fair in 12 patients from the nonoperative group and in no patients from the surgical group. Excellent results were not observed in any patient from the nonoperative group and were observed in 21 patients from the surgical group (67.7%). The isokinetic evaluation at 60 deg/s showed a decrease in strength of 41.7% in the nonsurgical group and 14.3% for the surgical group, which was significant at $P < .05$.

Conclusion: Total ruptures of the PMM exhibit better outcomes with surgical treatment than with nonoperative treatment based on the Bak criteria in athletes.

Keywords: shoulder; general; bench press; pectoralis major muscle rupture; muscle injuries; cortical button

An increase in ruptures of the pectoralis major muscle (PMM) has occurred in proportion to the number of people who are joining gyms and participating in weight-lifting events. In many cases, a lack of appropriate guidance regarding personal limitations and the abuse of anabolic steroids have caused these athletes to sustain total ruptures of the PMM.[14,25]

Failure to swiftly recognize the lesion on the part of health care professionals and insufficient guidance given to these athletes with respect to surgical correction have led, in most of these cases, to chronic lesions[2,3,15,18,21,40] and visible retraction of the ruptured PMM. Although some authors have reported better functional results from surgery during acute and chronic stages, we believe that PMM ruptures should be treated as soon as possible, similar to ruptures of the Achilles tendon or the distal biceps brachii.[26,29,32]

Early recognition of the clinical signs of PMM ruptures by orthopaedic surgeons, physical therapists, and physical trainers may prove to be critical for patient access to surgical treatment while the injury is still in the acute phase. The goal of our study was to confirm the results of our earlier study of the superiority of surgical treatment to

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1.17 Apêndice 2: de Figueiredo et al., 2014

Rare disease

CASE REPORT

Complex shoulder injuries in sports

Eduardo Antônio de Figueiredo, Paulo Santoro Belangero, Benno Ejnisman, Alberto de Castro Pochini

CETE—Centro de Traumatologia do Esporte, Universidade Federal de São Paulo, São Paulo, Brazil

Correspondence to
Dr Benno Ejnisman, bennoale@uol.com.br

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SUMMARY
A 26-year-old Olympic wrestling athlete presented with a pectoralis major muscle injury, glenohumeral instability and acromioclavicular joint dislocation separately. The patient underwent surgical treatment to repair these injuries. The pectoralis major muscle was reconstructed with a semitendinosus tendon graft using the endobutton technique, as described by Pochini *et al*. Treatment of the traumatic anterior instability was performed using the technique described by Bristow-Latarjet, and the acromioclavicular joint dislocation was repaired using the modified technique of Weaver-Dunn with the aid of an anchor. The athlete exhibited a rapid recovery and could return to normal activities 6 months after surgery. At present, 18 months postoperatively, the patient is asymptomatic.

Figure 1 Physical examination of patient, showing chronic rupture of the pectoralis major tendon.

BACKGROUND

By the end of the 1970s, only 45 cases of complete lesion of the pectoralis major muscle were described in the literature,[1] and at present, approximately 200 cases have been described.[2]

However, the occurrence of muscle lesions has not been associated with rupture of the pectoralis major muscle until now.

The present study aimed to describe the treatment provided to a competitive Olympic wrestling athlete presented with two injuries associated with pectoralis major muscle lesions, namely glenohumeral instability and acromioclavicular joint dislocation.

CASE PRESENTATION

A 26-year-old Olympic wrestling athlete presented with a sudden pain in the area of the pectoralis major muscle of the right shoulder after abduction and external rotation 6 months prior to the first consultation. Two years prior to that, the patient fell and suffered a trauma of the right shoulder. Since then, the trauma evolved to acromioclavicular joint dislocation grade III. Four years prior to that, the patient suffered the first episode of traumatic glenohumeral joint dislocation during training, which was followed by four episodes of dislocation of the same shoulder. These diagnoses were later confirmed by imaging (figures 1–9; videos 1 and 2).

TREATMENT

The patient immediately underwent surgical treatment to treat the aforementioned injuries. The reconstruction of the pectoralis major muscle was performed with a semitendinosus tendon graft using the endobutton technique, as described by Pochini *et al*[3] (figures 10 and 11;videos 3–5).

The treatment of the traumatic anterior instability was performed using the technique described by Bristow-Latarjet, which is performed with grafts that are removed from the coracoid process and fixed in the anterior margin of the glenoid with two screws (figures 12 and 13). The acromioclavicular joint dislocation was treated using the modified technique of Weaver-Dunn, with the aid of an anchor and a Kirschner wire (figures 14–16). The athlete showed a rapid recovery and returned to normal activities 6 months after the surgery. At present, 18 months postoperatively, the patient is asymptomatic (figures 17 and 18).

OUTCOME AND FOLLOW-UP

After the procedure, the patient remained immobilised for 6 weeks, and the Kirschner wire used for treating the acromioclavicular joint dislocation was removed at this time. Following the 6-week period,

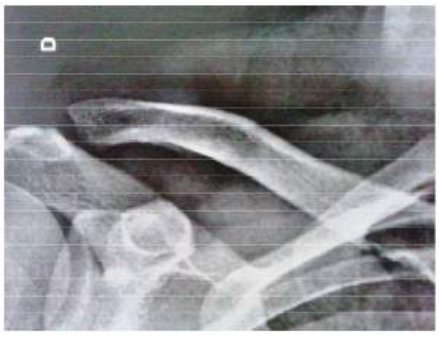

Figure 2 X-ray showing acromioclavicular dislocation in the right shoulder.

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1.18 Apêndice 3: Zogaib et al., 2014

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ORIGINAL ARTICLE

MINIMAL INVASIVE OSTHEOSINTESIS FOR TREATMENT OF DIAPHISEAL TRANSVERSE HUMERAL SHAFT FRACTURES

RODRIGO KALLÁS ZOGAIB[1], STEVEN MORGAN[2], PAULO SANTORO BELANGERO[3], HÉLIO JORGE ALVACHIAN FERNANDES[3], WILLIAM DIAS BELANGERO[1], BRUNO LIVANI[1]

ABSTRACT

Objective: To evaluate patients with transverse fractures of the shaft of the humerus treated with indirect reduction and internal fixation with plate and screws through minimally invasive technique. Methods: Inclusion criteria were adult patients with transverse diaphyseal fractures of the humerus closed, isolated or not occurring within 15 days of the initial trauma. Exclusion criteria were patients with compound fractures. Results: In two patients, proximal screw loosening occurred, however, the fractures consolidated in the same mean time as the rest of the series. Consolidation with up to 5 degrees of varus occurred in five cases and extension deficit was observed in the patient with olecranon fracture treated with tension band, which was not considered as a complication. There was no recurrence of infection or iatrogenic radial nerve injury. Conclusion: It can be concluded that minimally invasive osteosynthesis with bridge plate can be considered a safe and effective option for the treatment of transverse fractures of the humeral shaft. ***Level of Evidence III, Therapeutic Study.***

Keywords: Fracture fixation, internal. Surgical procedures, operative. Arm. Upper extremity.

Citation: Zogaib RK, Morgan S, Belangero PS, Fernandes HJA, Belangero WD, Livani B. Minimal invasive ostheosintesis for treatment of diaphiseal humeral shaft fractures. Acta Ortop Bras. [online]. 2014;22(2):94-8. Available from URL: http://www.scielo.br/aob.

INTRODUCTION

From the years 1980, the development of minimally invasive techniques began to draw interest in Brazil for treatment of diaphysis fractures of long bones, especially the femur.[1] The concept currently known as minimally invasive osteosynthesis with plates (MIOP) is based on the relative stability of the fracture with minimal damage to the surrounding soft tissues. Relative stability of the fracture secondary promotes healing, and subsequent formation of the bone callus, and reduces the possibility of infection and non-union.[2-7]

MIOP techniques were initially recommended for the treatment of comminuted fractures, because they promote a biological fixation without devitalization of bone fragments.[2-7] In the past 10 years, these principles have been widely used in the treatment of fractures of the humeral shaft, with good results.[8-15]

The MIOP technique is best used for the treatment of fractures with low strain.[2-4,7]

In the humerus, this technique has brought good clinical results, even in simple fractures traits. The aim of this study was to report the results of application of the minimally invasive technique with bridging plates in the treatment of transverse fractures of the humeral shaft analyzing the time of consolidation and the function.

PATIENTS AND METHODS

Between November 2000 and April 2011, adult patients with transverse fractures of the humeral shaft underwent reduction and fixation using the MIOP technique up to 15 days after the initial trauma. Exclusion criteria were compound fractures, pathological fractures, time over 15 days of the initial trauma, associated neurovascular injury, and the presence of open growth plate. The inclusion criterion was the presence of transverse diaphysis fracture of the humerus treated by the technique MIOP.

All fractures were operated with the same technique described below, and rehabilitation was performed following the same protocol.

The follow-up period ranged from 6 to 126 months (mean 51.6 months). DASH score was used in the evaluation of all patients during postoperative follow-up period.[15]

Surgical technique

The patient is kept in supine position on a standard operating

All the authors declare that there is no potential conflict of interest referring to this article.

1. Department of Orthopedics and Traumatology, Hospital das Clínicas, Universidade de Campinas (Unicamp), Campinas, SP, Brazil.
2. Mountain Orthopaedic Trauma Surgeons (MOTUS), Swedish Medical Center, Englewood, Colorado, USA.
3. Departament of Orthopedics and Traumatology, Universidade Federal de São Paulo (UNIFESP) São Paulo, SP, Brazil.

Word developed at Departament of Orthopedics and Traumatology, Universidade de Campinas (Unicamp), Campinas, SP, Brazil.
Corresponding author: Av. Washington Luís, 477 ap. 81, Santos, SP, Brazil. 11055-001. rzogaib@hotmail.com ou zkr1973@gmail.com

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1.19 Apêndice 4: Leal et al., 2014

OPEN ACCESS Freely available online

PLOS ONE

Identification of Suitable Reference Genes for Gene Expression Studies of Shoulder Instability

Mariana Ferreira Leal[1,2]*, Paulo Santoro Belangero[1], Carina Cohen[1], Eduardo Antônio Figueiredo[1], Leonor Casilla Loyola[1,2], Alberto Castro Pochini[1], Marília Cardoso Smith[2], Carlos Vicente Andreoli[1], Sintia Iole Belangero[2,3], Benno Ejnisman[1], Moises Cohen[1]

1 Departamento de Ortopedia e Traumatologia, Universidade Federal de São Paulo, São Paulo, São Paulo, Brazil, **2** Disciplina de Genética, Departamento de Morfologia e Genética, Universidade Ferderal de São Paulo, São Paulo, São Paulo, Brazil, **3** Laboratório Interdisciplinar de Neurociência Clínica, Departamento de Psiquiatria, Universidade Federal de São Paulo, São Paulo, São Paulo, Brazil

Abstract

Shoulder instability is a common shoulder injury, and patients present with plastic deformation of the glenohumeral capsule. Gene expression analysis may be a useful tool for increasing the general understanding of capsule deformation, and reverse-transcription quantitative polymerase chain reaction (RT-qPCR) has become an effective method for such studies. Although RT-qPCR is highly sensitive and specific, it requires the use of suitable reference genes for data normalization to guarantee meaningful and reproducible results. In the present study, we evaluated the suitability of a set of reference genes using samples from the glenohumeral capsules of individuals with and without shoulder instability. We analyzed the expression of six commonly used reference genes (*ACTB*, *B2M*, *GAPDH*, *HPRT1*, *TBP* and *TFRC*) in the antero-inferior, antero-superior and posterior portions of the glenohumeral capsules of cases and controls. The stability of the candidate reference gene expression was determined using four software packages: NormFinder, geNorm, BestKeeper and DataAssist. Overall, *HPRT1* was the best single reference gene, and *HPRT1* and *B2M* composed the best pair of reference genes from different analysis groups, including simultaneous analysis of all tissue samples. GenEx software was used to identify the optimal number of reference genes to be used for normalization and demonstrated that the accumulated standard deviation resulting from the use of 2 reference genes was similar to that resulting from the use of 3 or more reference genes. To identify the optimal combination of reference genes, we evaluated the expression of *COL1A1*. Although the use of different reference gene combinations yielded variable normalized quantities, the relative quantities within sample groups were similar and confirmed that no obvious differences were observed when using 2, 3 or 4 reference genes. Consequently, the use of 2 stable reference genes for normalization, especially *HPRT1* and *B2M*, is a reliable method for evaluating gene expression by RT-qPCR.

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Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper.

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Competing Interests: The authors have declared that no competing interests exist.

* Email: mariana.morf@epm.br

Introduction

Shoulder dislocation occurs in 1 to 2% of the population [1], and traumatic injuries account for 95% of shoulder dislocation episodes [2]. These shoulder injuries are frequently observed in young athletes that are involved in competitive sports [3], and shoulder instability (SI) is often observed after the initial episode of shoulder dislocation, with a recurrence rate of up to 100% in young athletes [4,5].

After episodes of shoulder dislocation, SI patients present plastic deformation of the glenohumeral capsule [6,7]. Although the antero-inferior (AI) region of the capsule is the most frequently injured site [7,8], previous macroscopic analysis of the collagen fiber bundle architecture in the AI region of the glenohumeral capsule revealed that a system of bundles spirally crossing one another permits the entire capsule to resist tensile and shear loads [9]. As a result, there is a reciprocal load-sharing relationship within the capsule whereby tensile load in either the anterior or superior structures is concomitant with laxity in the posterior (P) or inferior portion, respectively [7], suggesting that different portions of the capsule may be modified in traumatic anterior SI cases.

Currently, little is known about capsule biology, especially in patients with SI. An improved understanding of the underlying biology will be important for guiding patient management and development of new therapeutic options that will be complementary to surgery. Our group recently began investigating alterations in gene expression in SI, as gene expression analysis has previously been used to increase understanding of the molecular events involved in other traumatic sport injuries such as ligament [10,11] and tendon injuries (for a review, see [12]).

As a result of its accuracy, sensitivity and capacity for high throughput analysis, reverse-transcription quantitative polymerase

1.20 Apêndice 5: Leal et al., 2015

PLOS | ONE

RESEARCH ARTICLE

Identification of Suitable Reference Genes for Gene Expression Studies in Tendons from Patients with Rotator Cuff Tear

Mariana Ferreira Leal[1,2]*, Paulo Santoro Belangero[1], Eduardo Antônio Figueiredo[1], Carina Cohen[1], Leonor Casilla Loyola[1,2], Carlos Vicente Andreoli[1], Marília Cardoso Smith[2], Alberto de Castro Pochini[1], Benno Ejnisman[1], Moises Cohen[1]

1 Departamento de Ortopedia e Traumatologia, Universidade Federal de São Paulo, São Paulo, SP, Brazil, 2 Disciplina de Genética, Departamento de Morfologia e Genética, Universidade Federal de São Paulo, São Paulo, SP, Brazil

* mariana.morf@epm.br

Abstract

Rotator cuff tear is one of the most common causes of shoulder dysfunction. Gene expression analysis may be a useful tool for understanding tendon tears and the failure of cuff healing, and reverse-transcription quantitative polymerase chain reaction (RT-qPCR) has become an effective method for such studies. However, this technique requires the use of suitable reference genes for data normalization. Here, we evaluate the suitability of six reference genes (*18S*, *ACTB*, *B2M*, *GAPDH*, *HPRT1* and *TBP*) using samples from the rotator cuff tendons of 28 individuals with tendon tears (3 tendons regions) and 8 controls (2 tendon regions); for the tear patients, we evaluated ruptured and non-ruptured tendon samples. The stability of the candidate reference genes was determined using the NormFinder, geNorm, BestKeeper and DataAssist software packages. Overall, *HPRT1* was the best single reference gene, and *HPRT1+TBP* composed the best pair and *HPRT1+TBP+ACTB* composed the best trio of reference genes from the analysis of different groups, including the simultaneous analysis of all tissue samples. To identify the optimal combination of reference genes, we evaluated the expression of *COL1A1* and *COL3A1*, and no obvious differences were observed when using 2, 3 or 4 reference genes for most of the analyses. However, *COL3A1* expression differed between ruptured and non-ruptured (posterior superior region) tendons of patients only when normalized by *HPRT1+TBP+B2M* and *HPRT1+TBP*. On the other hand, the comparison between these two groups using the best trio of reference genes (*HPRT1+TBP+ACTB*) and 4 reference genes did not revealed a significant difference in *COL3A1* expression. Consequently, the use of suitable reference genes for a reliable gene expression evaluation by RT-qPCR should consider the type of tendon samples investigated. *HPRT1+TBP+ACTB* seems to be the best combination of reference genes for the analysis of involving different tendon samples of individuals with rotator cuff tears.

OPEN ACCESS

Citation: Leal MF, Belangero PS, Figueiredo EA, Cohen C, Loyola LC, Andreoli CV, et al. (2015) Identification of Suitable Reference Genes for Gene Expression Studies in Tendons from Patients with Rotator Cuff Tear. PLoS ONE 10(3): e0118821. doi:10.1371/journal.pone.0118821

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1.21 Apêndice 6: Figueiredo et al., 2016

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ORIGINAL ARTICLE

Rodeo athletes: management of shoulder instability

Eduardo A. FIGUEIREDO *, Paulo S. BELANGERO, Carina COHEN, Rafael L. LOUCHARD, Bernardo B. TERRA, Alberto C. POCHINI, Carlos V. ANDREOLI, Moisés COHEN, Benno EJNISMAN

Center of Sports Traumatology (CETE), Department of Orthopedics and Traumatology, São Paulo Federal University (UNIFESP), São Paulo, Brazil

*Corresponding author: Eduardo A. Figueiredo, Center of Sports Traumatology (CETE), Department of Orthopedics and Traumatology, São Paulo Federal University (UNIFESP), Rua Ouvidor Peleja 98, Vila Mariana, São Paulo, 04128-000 SP, Brazil. E-mail: eduardoafigueiredo@terra.com.br

ABSTRACT

BACKGROUND: The aim of this study was to describe epidemiological data and evaluate the clinical results of traumatic anterior glenohumeral instability in rodeo athletes.
METHODS: Thirteen patients, all male, with a mean age of 23.2 (18-31) years old, with anterior glenohumeral instability were include in this study. In 9 patients, the right side was affected. The mean time elapsed between injury and undergoing surgery was 56 months (24-120 months). The surgical technique used (arthroscopic or open bone block procedure) was chosen based on the ISIS (Instability Severity Index Score). Only professional athletes who had been in the sport for at least 60 months were included. Functional evaluation was conducted using the UCLA scale, after a 24-month follow-up period.
RESULTS: The number of dislocation episodes varied from 10 to 100 (mean 27 episodes). All of the patients were submitted a surgical treatment open bone block procedure, due to their degree of sport participation, type of sport (forced overhead and collision) and the presence of associated bone defect lesions. According to UCLA criteria, the results were excellent in 12 patients and good in one. The mean time elapsed before returning to the sport was five months, varying between two and ten months. Complications included one patient developing axillary neuropraxia, which was completely resolved six months after the operation, and another patient developed a superficial skin infection.
CONCLUSIONS: The rodeo athletes with anterior shoulder instability had serious associated bony lesions and has good outcome after bone block procedure.

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The combination of large untamed animals, young athletes, culture, fame, a high level of competitiveness and the potential financial gain makes rodeo an exciting sport with a high risk of injury as it is not seen in other sports.[1]

Rodeos are widespread and very popular in countries such as Brazil, the United States, Canada, Australia and New Zealand. There are different disciplines within the sport, the most common being bull riding.[2, 3]

The level of skill and strength required in rodeo is comparable to that of other sports; however, the inherent risk of injury, including fatal injury, is greater.[4-9]

Although this is a popular sport, practiced by a large number of individuals, there are few publications on the topic. As of yet, there seem to be no studies in the literature analyzing glenohumeral instability in rodeo athletes. Thus, this study aims to describe epidemiological data and evaluate clinical results of treating traumatic anterior glenohumeral instability in these athletes.

Materials and methods

All patients have given their informed consent for participation in the research study. Thirteen patients with anterior glenohumeral instability were treated between April 2010 and February 2012. All of the athletes

1.22 Apêndice 7: Carvalho et al., 2015

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Original Article

Partial rotator cuff injury in athletes: bursal or articular?☆

Cassiano Diniz Carvalho, Carina Cohen, Paulo Santoro Belangero, Eduardo Antônio Figueiredo, Gustavo Cará Monteiro, Alberto de Castro Pochini, Carlos Vicente Andreoli, Benno Ejnisman*

Centro de Traumatologia do Esporte (CETE), Department of Orthopedics and Traumatology, Universidade Federal de São Paulo, São Paulo, SP, Brazil

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ABSTRACT

A painful shoulder is a very common complaint among athletes, especially in the case of those in sports involving throwing. Partial lesions of the rotator cuff may be very painful and cause significant functional limitation to athletes' sports practice. The incidence of partial lesions of the cuff is variable (13–37%). It is difficult to make the clinical and radiological diagnosis, and this condition should be borne in mind in the cases of all athletes who present symptoms of rotator cuff syndrome, including in patients who are diagnosed only with tendinopathy.

Objective: To evaluate the epidemiological behavior of partial lesions of the rotator cuff in both amateur and professional athletes in different types of sports.

Methods: We evaluated 720 medical files on athletes attended at the shoulder service of the Discipline of Sports Medicine at the Sports Traumatology Center, Federal University of São Paulo. The majority of them were men (65%). Among all the patients, 83 of them were diagnosed with partial lesions of the rotator cuff, by means of ultrasonography or magnetic resonance, or in some cases using both. We applied the binomial test to compare the proportions found.

Result: It was observed that intra-articular lesions predominated (67.6%) and that these occurred more frequently in athletes in sports involving throwing (66%). Bursal lesions occurred in 32.4% of the athletes, predominantly in those who did muscle building (75%).

Conclusion: Intra-articular lesions are more frequent than bursal lesions and they occur predominantly in athletes in sports involving throwing, while bursal lesions were more prevalent in athletes who did muscle building.

☆ Work developed in Hospital São Paulo, Universidade Federal de São Paulo, São Paulo, SP, Brazil.

* *Corresponding author.*
E-mail: cassianodiniz78@gmail.com (C.D. Carvalho).

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1.23 Apêndice 8: Arliani et al., 2015

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Original Article

Acromioclavicular dislocation: treatment and rehabilitation. Current perspectives and trends among Brazilian orthopedists☆

*Gustavo Gonçalves Arliani**, *Artur Yudi Utino*, *Eduardo Misao Nishimura*, *Bernardo Barcellos Terra*, *Paulo Santoro Belangero*, *Diego Costa Astur*

Centro de Traumatologia do Esporte (Cete), Departamento de Ortopedia e Traumatologia, Universidade Federal de São Paulo (Unifesp), São Paulo, SP, Brazil

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ABSTRACT

Objective: To evaluate the approaches and procedures used by Brazilian orthopedic surgeons in treatment and rehabilitation of acromioclavicular dislocation of the shoulder.
Methods: A questionnaire comprising eight closed questions that addressed topics relating to treatment and rehabilitation of acromioclavicular dislocation was applied to Brazilian orthopedic surgeons over the three days of the 45th Brazilian Congress of Orthopedics and Traumatology, in 2013.
Results: A total of 122 surgeons completely filled out the questionnaire and formed part of the sample analyzed. Most of them came from the southeastern region of the country. In this sample, 67% of the participants would choose surgical treatment for patients with grade 3 acromioclavicular dislocation. Regarding the preferred technique for surgical treatment of acute acromioclavicular dislocation, a majority of the surgeons used subcoracoid ligature with acromioclavicular fixation and transfer of the coracoacromial ligament (25.4%). Regarding complications found after surgery had been performed, 43.4% and 32.8% of the participants, respectively, stated that residual deformity of the operated joint and pain were the complications most seen during the postoperative period.
Conclusions: Although there was no consensus regarding the treatment and rehabilitation of acromioclavicular dislocation, evolution had occurred in some of the topics analyzed in this questionnaire applied to Brazilian orthopedists. However, further controlled prospective studies are needed in order to evaluate the clinical and scientific benefit of these trends.

☆ Work developed at the Centro de Traumatologia do Esporte (CETE), Department of Orthopedics and Traumatology, Universidade Federal de São Paulo (UNIFESP), São Paulo, SP, Brazil.

* *Corresponding author.*
E-mail: ggarliani@hotmail.com (G.G. Arliani).

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1.24 Apêndice 9: Vieira et al., 2015

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Original Article

Rotator cuff injuries: current perspectives and trends for treatment and rehabilitation☆

Fabio Antonio Vieira, Paul Juma Olawa, Paulo Santoro Belangero, Gustavo Gonçalves Arliani, Eduardo Antônio Figueiredo, Benno Ejnisman*

Escola Paulista de Medicina, Universidade Federal de São Paulo (UNIFESP), São Paulo, SP, Brazil

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ABSTRACT

Objective: To map out the approaches used by Brazilian orthopedists in treating complete tears of the rotator cuff.
Methods: A multiple-choice questionnaire was handed out to 232 orthopedists at the 45th Brazilian Congress of Orthopedics and Traumatology. Of these, 207 were returned but five were incomplete and were excluded. Thus, 202 questionnaires were used.
Results: Among the orthopedists who answered the questionnaires, around 60% were from the southeastern region and 46% were shoulder and elbow surgeons. There was a significant association ($p < 0.05$) between length of experience and number of rotator cuff repairs performed per year. There was also a significant association ($p < 0.05$) between shoulder specialty and the following variables: arthroscopic technique, use of anchors in a single-row configuration, mean time taken for an indication for surgery to be made in cases of traumatic and degenerative lesions, use of a specific protocol for postsurgical rehabilitation, return to sport and indication of irreparable injuries.
Conclusions: Brazilian shoulder surgeons have well-established approaches toward treating rotator cuff injuries. Most of these approaches differ significantly from those of other specialties. This shows the importance of placing value on training in preparing shoulder specialists in this country.

☆ Work performed in the Sports Traumatology Group, Orthopedics and Traumatology Service, Escola Paulista de Medicina, Universidade Federal de São Paulo (UNIFESP), São Paulo, SP, Brazil.

* *Corresponding author.*
E-mail: fabioavepm74@gmail.com (F.A. Vieira).

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1.25 Apêndice 10: Andreoli et al., 2016

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Case Report

Tendon of the long head of the biceps originating from the rotator cuff – An uncommon anatomical variation: case report☆

Carlos Vicente Andreoli, Leonardo Roure Esteves, Eduardo Figueiredo, Paulo Santoro Belangero, Alberto de Castro Pochini, Benno Ejnisman*

Universidade Federal de São Paulo (Unifesp), São Paulo, SP, Brazil

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ABSTRACT

Anatomical variations at the origin of the biceps tendon have been described by several authors, but occurrences of an origin in the supraspinatus are rare. It is unclear whether this variation might contribute toward pathological conditions of the shoulder. Our objective here was to describe a case of an anatomical variation in the origin of the tendon of the long head of the biceps.

The clinical information, preoperative images and arthroscopic images relating to a patient with an aberrant origin of the long head of the biceps, which was observed during shoulder arthroscopy, were reviewed.

In this case study, the origin of the biceps was found in the rotator cuff, without any origin from the supraglenoid tubercle or upper labrum. This variant did not seem to contribute toward the pathological condition of the shoulder, and standard treatment for the concomitant condition was sufficient for treating it.

Tendão da cabeça longa do bíceps originado do manguito rotador – Uma variação anatômica incomum: relato de caso

Palavras-chave:
Tendões
Ombro
Bainha rotadora

RESUMO

As variações anatômicas na origem do tendão do bíceps foram descritas por vários autores, mas a ocorrência de sua origem no supraespinhal é rara. Não está claro se essa variação pode contribuir para condições patológicas do ombro. Nosso objetivo é descrever um caso de uma variação anatômica da origem da cabeça longa do tendão do bíceps.

☆ Work performed in the Discipline of Sports Medicine, Department of Orthopedics and Traumatology, Escola Paulista de Medicina (EPM), Universidade Federal de São Paulo (UNIFESP), São Paulo, SP, Brazil.

* *Corresponding author.*
E-mail: andreolicruz@uol.com.br (C.V. Andreoli).

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