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Genetic and Other Intrinsic Factors Influencing Risk for Elbow Tendinopathy

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CERTIFICA:

Que la presente memoria, titulada "**Genetic and other intrinsic factors influencing risk for elbow tendinopathy**", corresponde al trabajo realizado bajo su dirección por Dña. **Emily Renae McPeek**, para su presentación como Tesis Doctoral en el Programa de Doctorado en Fisiología de la Universitat de València.

Y para que conste firma el presente certificado en Valencia, a 1 de noviembre de 2018.

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LIST OF ABBREVIATIONS USED IN THIS WORK

ANOVA	analysis of variance
ATP	adenosine triphosphate
BMI	body mass index
COMP	cartilage oligomeric matrix protein
CTGF	connective tissue growth factor
CVD	cardiovascular disease
DASH	disabilities of the arm, shoulder and hand
DNA	deoxyribonucleic acid
ECM	extracellular matrix
EDTA	ethylenediaminetetraacetic acid
GAG	glucosaminoglycan
HDL-C	high-density lipoprotein cholesterol
HWE	Hardy-Weinberg equilibrium
IGF-1	insulin-like growth factor 1
IL-1β	interleukin 1beta
IL-6	interleukin 6
ISAK	International Society for the Advancement of Kinanthropometry
LCL	lateral collateral ligament
LDL-C	low-density lipoprotein cholesterol
MAPK	mitogen-activated protein kinase
MCL	medial collateral ligament
MMP	matrix metalloproteinase

MSK	musculoskeletal
MTJ	myotendinous junction
NSAID	non-steroidal anti-inflammatory drug
OC	oral contraceptive
OTJ	osteotendinous junction
PCR	polymerase chain reaction
PG	proteoglycan
RNA	ribonucleic acid
SLRP	small leucine-rich protein
SNP	single nucleotide polymorphism
SOM	self-organizing map
TG	triglycerides
TGF-β	transforming growth factor beta
TNC	tenascin C
UCIM	Unidad Central de Investigación Médica
US	ultrasound
UTS	ultimate tensile strength
VEGF	vascular endothelial growth factor

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ABSTRACT AND RESUMEN

ABSTRACT

Many extrinsic and intrinsic factors have been attributed to increased risk of tendinopathy; however, most research has been done on the tendons of the lower limbs, and very few have examined risk factors for tendinopathy of the elbow. Recent research on tendinopathy has focused on the role of certain genetic polymorphisms in tendinopathy risk, especially genes involved in collagen synthesis and regulation. The aim of this study was to test for a relationship between certain collagen gene single nucleotide polymorphisms (SNPs) and elbow tendon pathology. In this case-control study, 137 young adult athletes whose sports participation involves loading of the upper limb were examined for the presence of structural abnormalities indicative of pathology in the tendons of the lateral and medial elbow using ultrasound imaging and genotyped for the following SNPs: COL5A1 rs12722, COL11A1 rs3753841, COL11A1 rs1676486, and COL11A2 rs1799907. Anthropometric measurements and data on participants' elbow pain and dysfunction were also collected. Participants in the structural abnormality group had significantly higher scores in pain and dysfunction. A significant relationship between COL11A1 rs3753841 genotype and elbow tendon pathology was found, with the CT variant associated with increased risk of pathology.

RESUMEN

Muchos factores de riesgo, tanto extrínsecos como intrínsecos, han sido relacionados con el riesgo de padecer la tendinopatía. Sin embargo, la mayoría de los estudios al respecto han investigado los tendones de las extremidades inferiores, y son muy pocos los estudios que investigan factores de riesgo en tendinopatía del codo. Últimamente, el enfoque de investigaciones en la tendinopatía ha sido el papel de algunos polimorfismos genéticos, sobre todo los genes involucrados en la síntesis y la regulación del colágeno. El objetivo del presente trabajo es averiguar si existe una relación entre algunos polimorfismos de un solo nucleótido (SNP por sus siglas en inglés) en genes de colágeno y el riesgo de patología de los tendones del codo. En este estudio de casos y controles, se examinaron mediante ecografía a 137 jóvenes deportistas cuya participación deportiva supone una carga de la extremidad superior para la presencia de irregularidades estructurales en los tendones laterales y mediales de los codos que implican una patología del tendón. Se llevó a cabo un análisis de genotipo para los siguientes SNP: COL5A1 rs12722, COL11A1 rs3853841, COL11A1 rs1676486 y COL11A2 rs1799907. Además, se recogieron medidas antropométricas y datos sobre el dolor y la discapacidad de los sujetos. Los sujetos en el grupo patológico presentaban puntuaciones significativamente más altas para el dolor y la discapacidad. Había una relación significativa entre el genotipo del COL11A1 rs3753841 y patología de los tendones del codo, siendo la variante CT asociado a mayor riesgo.

RESUMEN AMPLIO

Introducción y objetivos

La tendinopatía es una enfermedad común que afecta a una gran proporción de la población, formando un 30% de las lesiones musculoesqueletales, y es el trastorno más prevalente del tendón. Se define habitualmente como una lesión de sobreuso, que resulta en la degeneración del tendón como consecuencia de una desorientación y desorganización del colágeno sin una reacción inflamatoria, acompañada de dolor y pérdida de función. Se desconoce la incidencia exacta de la tendinopatía del codo, pero la incidencia aproximada es de entre un 1% y 3% de adultos cada año en los tendones laterales del codo, y de entre un 0.1% y 0.75% en los tendones mediales del codo.

En deportistas, las personas cuya práctica deportiva supone una carga del miembro superior representan un grupo de riesgo elevado de desarrollar tendinopatía del codo, sobre todo en los deportes como son el polo acuático, el tenis, la natación, el béisbol, o el golf, ya que este tipo de deporte implica el miembro superior en movimientos de tirar por encima de la cabeza o de pronación del antebrazo con flexión de la muñeca. Sin embargo, la tendinopatía del codo no se limita a los deportistas, y puede desarrollarse a través de diversas actividades rutinarias en el trabajo o en la vida diaria que suponen movimientos similares.

Se suele relacionar la tendinopatía del codo a un sobreuso crónico y movimientos repetitivos del mismo. No obstante, debido a la

respuesta diferenciada entre individuos frente la misma carga externa, ha habido un aumento en los últimos años en investigaciones sobre factores de riesgo intrínsecos. A día de hoy, varios estudios han descrito la contribución de factores genéticos en las tendinopatías del Aquiles y de la patela, pero los estudios sobre los tendones del miembro superior son escasos, con unos pocos investigando los factores genéticos en la tendinopatía del manguito rotor y solo un estudio sobre el llamado "codo de tenista".

El colágeno es el componente principal de la matriz extracelular (MEC) del tendón. Su función está relacionada con la formación de sustancias fibrilares y microfibrilares en la MEC, con un papel importante a la hora de determinar las propiedades específicas de cada tejido. Aunque el colágeno tipo I es el más abundante en el tendón, también hay cantidades variables de otros tipos de colágeno, incluidos los tipos II, III, V y XI. El colágeno tipo III es el tipo principal involucrado en la regulación del fibrilogénesis y la extensibilidad del tendón, mientras los tipos V y XI están asociados a los tipos I y II, respectivamente, influyendo en la cantidad y calidad de estos. La isoforma principal del tipo V es un heterotrímero formado por dos cadenas alfa-1 (codificadas por el gen COL5A1) y una cadena alfa-2 (codificada por el gen COL5A2), el cual intercala con el colágeno tipo I para modular el fibrilogénesis. La isoforma principal del tipo XI es un heterotrímero formado por una cadena alfa-1 y una cadena alfa-2 (COL11A1 y COL11A2, respectivamente), más una cadena alfa-2 del tipo II. Estas moléculas forman entrecruzamientos fuertes entre las

células del tendón además de ayudar a mantener la separación y el diámetro de las fibrillas del colágeno tipo II.

Factores de riesgo genéticos en la tendinopatía

Algunas mutaciones en los genes COL5A1, COL11A1 y COL11A2 han sido asociadas a trastornos que son resultado de una alteración o pérdida de función en el colágeno. Por ejemplo, unas mutaciones en el COL5A1 y en el COL11A1 son presentes en ciertos tipos del síndrome de Ehlers-Danlos y en el síndrome de Marshall, respectivamente. Mutaciones en el COL11A2 han sido asociadas a la displasia otoespondilomegaepifisaria y el síndrome de Weissenbacher-Zweymüller. Tanto el COL11A1 como el COL11A2 han sido asociados al síndrome de Stickler. Todos estos trastornos pueden impactar en la extensibilidad de las articulaciones o provocar anormalidades esqueletales debido a una alteración del colágeno.

Se ha estudiado los alelos del polimorfismo de un solo nucleótido (SNP, de single nucleotide polymorphism) rs12722 del gen COL5A1 (citosina, C, o timina, T) con respecto a la tendinopatía en investigaciones previas. Mientras el genotipo CC ha sido asociado a un menor riesgo de tendinopatía crónica del Aquiles, el genotipo CT ha sido asociado a una elasticidad menor en el miembro inferior, y el genotipo TT es asociado a un menor rango de movilidad, sobre todo en las personas de edad avanzada, y a un mayor riesgo de tendinopatía crónica del Aquiles. Altinisik et al. (2015) encontraron una asociación significativa entre las variantes COL5A1 rs12722 y rs 13946 y el riesgo de tendinopatía del epicóndilo lateral (codo de tenista). Los autores

registraron un efecto protector del genotipo CC en el desarrollo de la tendinopatía del codo en el caso del SNP rs12722, y del genotipo TT en el caso del SNP rs13946.

Se ha estudiado también algunas variantes de los genes COL11A1 y A2 con respecto a la tendinopatía, aunque en un grado menor. Hay et al. (2013) investigó COL11A1 rs3753841 (T/C), COL11A1 rs1676486 (C/T) y COL11A2 rs1799907 (T/A) para averiguar si existía una asociación independiente con tendinopatía crónica del Aquiles, sin hallar dicha relación. Sin embargo, registraron una sobrerrepresentación en el grupo patológico de ciertos seudo haplotipos inferidos, que consistían en combinaciones de estos polimorfismos, y también de seudo haplotipos de los polimorfismos del colágeno XI en combinación con el genotipo del COL5A1 rs7174644 (-/AGGG).

Además de los factores de riesgo de carácter genético, también hay numerosos estudios sobre otros factores de riesgo intrínsecos que pueden influir en la tendinopatía. Por ejemplo, el riesgo de padecer afecciones del tendón aumenta con el envejecimiento debido a cambios en la estructura y biomecánica de los tendones conforme avanza la edad. Otro factor de riesgo de mucho estudio es la obesidad y el sobrepeso. En líneas generales, las personas con un nivel elevado de adiposidad presentan anormalidades en los tendones con mayor incidencia que las personas con un IMC normal. El mecanismo por el cual el sobrepeso aumenta el riesgo de padecer una lesión tendinosa parece tener múltiples facetas tanto mecánicas como metabólicas.

De modo similar, en 2009, Longo et al. registró una relación entre niveles elevados de glucosa en la sangre y el riesgo de padecer una rotura de los tendones del manguito rotador, aunque el número de estudios en este respecto es muy bajo en comparación con los estudios sobre la relación entre la diabetes y la tendinopatía.

Objetivos del presente estudio

Por todo lo anteriormente descrito con respecto a la relación entre factores intrínsecos, tanto genéticos como no genéticos, y la tendinopatía, los objetivos del presente estudio son los siguientes:

1. Analizar la influencia de los SNP COL5A1 rs12722, COL11A1 rs3753841, COL11A1 rs1676484 y COL11A2 rs1799907 en el riesgo de padecer tendinopatía del codo.
2. Analizar la influencia de otras variables demográficas y antropométricas, como son el seco, composición corporal, IMC, glucemia, y laxitud de las articulaciones en la tendinopatía del codo.
3. Analizar la influencia de ciertos factores de estilo de vida en el riesgo de padecer tendinopatía del codo.
4. Averiguar la importancia relativa de cada uno de estos factores con respecto al riesgo de padecer tendinopatía del codo.

Material y metodología

Se seleccionó una muestra de 137 sujetos (edad media 23 ± 5,5 años, 77 hombres y 60 mujeres) de la población general según su

participación en deportes que implican una carga del miembro superior. Los criterios de inclusión fueron: edades comprendidas entre 18 y 50 años, participación semanal en su deporte de al menos 4 horas, y participación continua en su deporte durante al menos 2 años. Fueron excluidos aquellos sujetos que cumplieron alguno de los criterios de exclusión: historial de cirugía u osteoartritis del codo, historial de subluxación o dislocación del codo, fractura del húmero, radio o ulna, tomar medicamentos que pueden alterar las propiedades del tendón durante los últimos 6 meses, embarazo, o inflamación del codo como, por ejemplo, artritis reumatoide o espondilitis anquilosante.

El protocolo del estudio fue aprobado por el Comité de Ética de Investigación en Humanos de la Universidad de Valencia de acuerdo con los principios establecidos en la Declaración de Helsinki relativo a los derechos humanos, y cumplía los requisitos establecidos en la legislación española en el ámbito de la investigación biomédica, la protección de datos de carácter personal, y la bioética.

Cuestionarios y recolección de datos

Se recogió datos demográficos (sexo, edad, lado dominante y grupo étnico) y datos sobre los hábitos, el historial clínico y antecedentes familiares, y la participación deportiva de cada sujeto. Los sujetos rellenaron cuestionarios estandardizados para cuantificar su nivel de dolor y funcionalidad, incluidos el DASH (Disabilities of the Arm, Shoulder and Hand) general, deporte y ocupacional y el Shoulder Activity Level Questionnaire. La laxitud de las articulaciones fue evaluada mediante el test de Beighton, se llevó a cabo una prueba

para valorar el rango de movilidad del codo, y se recogieron datos antropométricos: altura, peso, IMC, circunferencia de la cintura y porcentaje grasa corporal.

El DASH es un cuestionario específico que evalúa la calidad de vida relacionada con los problemas del brazo, hombro y mano; es decir, del miembro superior. Consiste en tres apartados con una puntuación del 1 a 5 sobre la posibilidad de realizar actividades de la vida diaria, donde una puntuación mayor indica mayor percepción de la intensidad de los síntomas. El primer apartado, "General", fue obligatorio para todos los sujetos y contiene 30 preguntas. Los otros dos apartados, "Trabajo/Ocupación" y "Atletas/Músicos de Alto Rendimiento", contienen 4 preguntas cada uno. La puntuación total del apartado general puede oscilar entre 30 y 150 puntos. Esta puntuación se transforma en una escala de 0 a 100 (mejor a peor puntuación posible). Los otros dos apartados se puntúan por separado según el mismo método.

El cuestionario de nivel de actividad del hombro, a pesar de su nombre, es una herramienta que permite medir el uso de todo el miembro superior registrando el nivel de actividad máximo que ha alcanzado el sujeto en el último año. Para ello, se valoran 5 ítems relacionados a diferentes actividades exigentes para la articulación del hombro y la frecuencia con la que se realizaba a la semana, desde nunca a todos los días, con puntuaciones del 0 a 4 según la frecuencia. Se considera un nivel de actividad bajo a puntuaciones por debajo de 7, nivel medio a puntuaciones entre 7 y 14, y nivel alto a puntuaciones

más de 14. El segundo apartado corresponde al nivel de participación en actividades deportivas de contacto o que impliquen movimientos repetitivos por encima de la cabeza y el tipo de participación en función del nivel de competencia (no competitivo, federado, profesional).

El test de Beighton es un método sencillo para cuantificar la laxitud articular y la hipermovilidad. Utiliza un sistema de 9 puntos en función de la posibilidad de sobrepasar los límites normales de la articulación testada. El umbral que determina la laxitud articular se sitúa en 4 según las recomendaciones del propio test. Para valorar la movilidad del codo, se midió con un goniómetro en ángulo de flexión y extensión (en posición dorsal decubitis) y pronación y supinación (con el sujeto sentado).

En una hoja de registro de datos antropométricos, se registraron los siguientes datos: altura mediante la medida en máxima extensión con un tallímetro. El sujeto se colocó en bipedestación con los talones juntos y realizó una inspiración profunda, manteniendo la cabeza en el plano de Frankfort; peso y composición corporal medidos a través de un medidor corporal TANITA por medio de impedancia bioeléctrica donde se obtuvo el peso y el porcentaje graso. El sujeto se encontraba en ayunas de 10 horas, descalzos y sin calcetines, y se colocó en bipedestación con los brazos relajados y sosteniendo los agarres manteniendo las palmas de las manos en contacto con los 4 electrodos correspondientes; IMC calculado del peso en kilogramos y la altura en metros según la fórmula IMC = peso en kg / (altura en m)^2;

circunferencia de la cintura tomada a nivel intermedio entre el último arco costal y la cresta ilíaca en la posición más estrecha del abdomen.

Grupos y análisis de genotipos

Se asignó a los sujetos a dos grupos según la presencia o ausencia de patología en cualquiera de los tendones del codo. Para ello, se llevó a cabo una examinación ecográfica (FUJIFILM SonoSite NanoMax US system) por un fisioterapeuta especializado en evaluación ecográfica para comprobar la integridad de los tendones del codo. Se empleó un gel de base acuosa para favorecer la visibilidad. Se tomaron imágenes ecográficas de los tendones de ambos codos, y estos se clasificaron en función de completa normalidad o con presencia de alguna anormalidad (aumento en tamaño, hipoecogenicidad, evidencia de una rotura o una rotura parcial, etc.). Aquellos sujetos que presentaban cualquier anormalidad tendinosa en uno o ambos codos fueron asignados al grupo patológico, mientras aquellos que presentaban tendones normales fueron asignados al grupo control. Al final, 36 sujetos fueron asignados al grupo patológico y 101 al grupo control.

Para poder determinar los polimorfismos genéticos de cada sujeto y relacionarlos con la tendinopatía del codo, se tomó una muestra de sangre venosa de aproximadamente 4,5 ml mediante punción venosa de la vena del antebrazo y extraída en tubos EDTA Vacutainer. Los sujetos acudieron a la extracción tras un ayuno de 6 horas para una correcta medición de la glucosa en ayunas. Las muestras de sangre fueron almacenadas a 4°C durante el protocolo experimental hasta su

posterior transporte bajo condiciones ideales y almacenamiento a -80°C durante dos meses. Una vez terminada la fase de recolección de datos, todas las muestras fueron trasladadas a la Unidad de Genética y Biología Molecular en el Hospital Universitario La Ribera, donde se llevó a cabo el análisis genético.

La extracción del ADN de las muestras de sangre se realizó según los procedimientos estandarizados. Se emplearon la tecnología basada en la PCR (reacciones en cadena de la polimerasa) en tiempo real. La PCR en tiempo real es una técnica que combina la amplificación y la detección en un mismo paso correlacionando el producto de la PCR en cada ciclo con una señal de intensidad de fluorescencia. Para determinar el genotipo de los polimorfismos seleccionados en cada sujeto, se realizó un análisis de genotipificación de SNP TaqMan (Applied Biosystems, Foster City, California, Estados Unidos) con un sistema de PCR en tiempo real Fast7900HT (Applied Biosystems). Los resultados de la genotipificación fueron reproducidos para cada sujeto en tres pruebas independientes.

Análisis estadístico

Se empleó el software matemático Matlab®, el programa de libre distribución R, y el SPSS® como herramientas en el tratamiento de los datos del estudio. Para las variables cuantitativas, se usó el test de Wilcoxon-Mann-Whitney para buscar diferencias estadísticamente significativas entre el grupo patológico y el grupo control. Se usó el test de chi-cuadrado para buscar asociaciones entre las variables categóricas, como son los distintos genotipos, y la presencia de

tendinopatía del codo. También se empleó una regresión logística para controlar los efectos de ciertas variables intrínsecas que tienen una asociación demostrada con la patología tendinosa, como la edad y el IMC, para poder tener en cuenta un efecto potencialmente confuso de dichas variables a la hora de comprobar una posible asociación entre los genotipos de los SNP y la patología.

Para una mejor visualización y agrupación de los sujetos, se creó un mapa autoorganizado. Este es un tipo de red neuronal que permite representar de forma conjunta todas las variables de interés y todos los patrones entre ellas. Por último, para determinar la importancia relativa de las variables a la hora de predecir la pertenencia a un grupo u otro, se empleó el test de Random Forest, un método basado en aprendizaje máquina.

Resultados y conclusiones

Según la examinación ecográfica de la muestra en el presente estudio, el 73.72% de los sujetos presentaba un estado normal de todos los tendones del codo (sin tendinopatía), mientras el otro 26.28% presentaba cambios patológicos en alguno de los tendones del codo (tendinopatía). Por lo tanto, 36 sujetos fueron asignados al grupo patológico o caso, y 101 al grupo normal o control.

Se encontró asociaciones estadísticamente significativas entre algunas de las variables estudiadas, incluidos los factores genéticos, factores biológicos cuantitativos y factores categóricos no genéticos, además de algunas tendencias que, a pesar de que no fueran

estadísticamente significativas, sugieren que podría existir una relación. Las técnicas de aprendizaje máquina revelaron información adicional sobre las relaciones entre la tendinopatía del codo y las variables estudiadas, así como las relaciones entre las propias variables.

De los 36 sujetos en el grupo patológico, 10 presentaban anormalidades en el tendón extensor común (codo de tenista; 7 en el lado derecho, 2 en el lado izquierdo, y 1 bilateral), otros 16 presentaban anormalidades en el tendón flexor común (codo de golfista; 8 en el lado derecho, 4 en el lado izquierdo y 4 bilateral), y los 10 restante tenían alguna combinación de anormalidades tendinosas en el epicóndilo y tróclea.

Cuestionarios y test de funcionalidad

Las puntuaciones en los apartados “deportes” y “ocupacional” del DASH fueron mayores en el grupo patológico ($p=0.03$ y $p=0.005$, respectivamente), lo cual indica un nivel mayor de dolor y disfuncionalidad en dicho grupo frente a los controles. Entre las variables categóricas, según el análisis de chi-cuadrado, dos factores no genéticos se destacaron como variables con una relación significativa con la tendinopatía del codo: participación en deportes de lanzamiento y una historia de tendinopatía previa. En los deportes de lanzamiento, aquellos sujetos que participaban con un nivel de competencia federado tenían más posibilidades de padecer tendinopatía del codo frente a los que participaban a nivel no competitivo o a nivel profesional. En cuanto a la tendinopatía previa,

una tendinopatía previa en el lado dominante o bilateral fue asociada a un mayor riesgo de padecer una tendinopatía actual del codo.

Factores genéticos

COL5A1 rs12722: la frecuencia de los alelos para este SNP fue de 65.69% citosina / 34.31% timina para el primer alelo, y 8.76% citosina / 91.24% timina para el segundo alelo. La distribución de genotipos fue de 8.76% CC, 56.93% CT, 34.31% TT. Este SNP ha sido asociado al llamado codo de tenista en un estudio previo (Altinisik et al., 2015). Sin embargo, nuestro análisis de chi-cuadrado no reveló diferencias significativas entre el grupo patológico y el grupo normal con respecto al genotipo de COL5A1 rs12722. Un análisis posterior con mapas autoorganizados mostró una tendencia hacia una asociación para este SNP.

COL11A1 rs1676486: la frecuencia de los alelos para este SNP fue de 77.37% citosina / 22.63% timina para el primer alelo, y 97.08% citosina / 2.92% timina para el segundo alelo. La distribución de genotipos fue de 77.37% CC, 19.71% CT, 2.92% TT. En el análisis de chi-cuadrado, no se encontró diferencias significativas para el genotipo de este SNP entre el grupo patológico y el grupo control.

COL11A1 rs3753841: la frecuencia de los alelos para este SNP fue de 65.69% citosina / 34.31% timina para el primer alelo, y 16.79% citosina / 83.21% timina para el segundo alelo. La distribución de genotipos fue de 16.79% CC, 48.91% CT, 34.31% TT. El análisis de chi-cuadrado reveló una asociación estadísticamente significativa entre

el genotipo de este SNP y la presencia de tendinopatía del codo (p=0.025). Los sujetos con el genotipo CT tenían más posibilidades de padecer tendinopatía que los sujetos con los dos genotipos homocigotos. El genotipo de este SNP también fue asociado al otro SNP de COL11A1, el rs1676486 ($p=6.04 \times 10^{-8}$) a pesar de que este otro SNP no fue relacionado con la tendinopatía del codo. Por último, había una asociación significativa entre este SNP y un historial familiar de colesterol alto (p=0.027).

COL11A2 rs1799907: la frecuencia de los alelos para este SNP fue de 37.23% adenosina / 62.77% timina para el primer alelo, y 9.49% adenosina / 90.51% timina para el segundo alelo. La distribución de genotipos fue de 9.49% AA, 27.74% AT, 62.77% TT. En el análisis de chi-cuadrado, no se encontró diferencias significativas para el genotipo de este SNP entre el grupo patológico y el grupo control.

Otros factores intrínsecos

Algunos otros factores fueron asociados a la presencia de tendinopatía del codo de forma estadísticamente significativa. Entre ellos, los más destacados son el IMC (p=0.00002) y las variables relacionadas con ello, como son el porcentaje grasa corporal (p=0.00003), peso (p=0.001) y circunferencia de la cintura (p=0.003). No había diferencias entre los dos grupos con respecto a la composición de sexo o edad. Se empleó una regresión logística para controlar ciertas variables que han sido asociadas a la patología tendinosa en otros estudios. En el modelo de la regresión logística, la

relación entre el genotipo del SNP COL11A1 rs3753841 dejó de ser significativa cuando se controlaba los efectos del IMC.

Se creó mapas autoorganizados a partir de datos normalizados para poder visualizar colectivamente las distintas variables de interés y determinar su relación con la presencia de la tendinopatía del codo, y sus relaciones entre ellos. Este análisis reveló algunas tendencias que no salieron estadísticamente significativas en el chi-cuadrado; por ejemplo, el genotipo de los SNP COL5A1 rs12722 y COL11A2 rs1799907 con la presencia de tendinopatía del codo. Además de la aparente relación entre las variables y la patología, podemos inferir también relaciones entre las otras variables estudiadas. Por ejemplo, en mayor porcentaje de grasa corporal fue asociado a mayor laxitud y hipermovilidad, y un menor nivel de actividad del hombro.

Se llevó a cabo un Random Forest test para valorar la importancia relativa de cada variable estudiada. Las variables que mejor predecían la pertenencia al grupo patológico o normal fueron: porcentaje grasa corporal, IMC, nivel de actividad del hombro, y altura. Entre los factores genéticos, el genotipo del SNP COL11A1 rs3753841 tenía mayor importancia, seguido por el genotipo del COL11A2 rs1799907 y el genotipo del COL5A1 rs12722, aunque estos últimos apenas tenían más importancia que los factores menos importantes según este test (fumador/no fumador, lateralidad, historial familiar de diabetes y inestabilidad del codo derecho). De importancia intermedia, tenemos las medidas de hipermovilidad (supinación, flexión y pronación del codo derecho), edad, glucemia, y laxitud articular según el test de

Beighton. En líneas generales, los factores antropométricos tenían más importancia que los factores genéticos.

La etiología de la tendinopatía del codo es multifactorial, dado que hemos encontrado diversos factores que influyen en su presencia. Entre dichos factores, encontramos factores genéticos, así como factores antropométricos y puntuaciones de funcionalidad. Las conclusiones del presente trabajo son las siguientes:

1. El genotipo del SNP COL11A1 rs3753841 fue asociado a la incidencia de tendinopatía del codo (p=0.025); los sujetos en el grupo patológico tenían más probabilidades de tener el genotipo CT. Ninguno de los otros SNP estudiados mostraron una relación significativa. Sin embargo, hay una tendencia hacia una relación para los SNP COL11A2 rs1799907 y COL5A1 rs12722, como podemos ver en los mapas autoorganizados.

2. Se encontró una relación significativa entre otros factores intrínsecos y la presencia de tendinopatía del codo: peso (p=0.001), IMC (p=0.00002), circunferencia de la cintura (p=0.003) y porcentaje grasa corporal (p=0.00003). También puntuaciones en el cuestionario DASH en los apartados de deportes (p=0.03) y ocupacional (p=0.005), y una historia de tendinopatía previa bilateral o en el lado derecho (p=0.02). Otros factores mostraron una tendencia hacia una asociación, como son: menor estatura, mayor

nivel de glucosa en la sangre, hipermovilidad del codo derecho, y laxitud articular.

3. La participación en deportes de lanzamiento fue asociada a tendinopatía del codo (nivel de competencia federado, p=0.03). No se encontró una relación significativa entre la participación en deportes de contacto y la patología, pero sí que existía una relación entre participación en este tipo de deporte y la inestabilidad en ambos codos.

4. Según los resultados del Random Forest Test, los factores con mayor impacto en la patología de los estudiados son los antropométricos: porcentaje grasa corporal, IMC, nivel de actividad del hombro, y altura. Se mostró que los factores genéticos son estadísticamente menos importante que las variables hipermovilidad del codo, edad, glucemia, y laxitud articular, pero más importante que las variables fumador/no fumador, lateralidad, historial familiar de diabetes y, sorprendentemente, inestabilidad del codo. Los factores genéticos, de mayor a menor importancia, son: genotipo del SNP COL11A1 rs3753841 (estadísticamente significativa), COL11A2 rs1799907 y COL5A1 rs12722 (ninguno de los dos siendo estadísticamente significativa).

I. INTRODUCTION

1. Justification of the work

1.1. Tendinopathy: overview and epidemiology

Tendinopathy is a common condition that affects a large portion of the population. It is an umbrella term for clinical presentation of tendon pain during loading and upon palpation, sometimes accompanied by loss of function (McAuliffe, 2016). It is generally defined as an overuse injury resulting in tendon degeneration due to collagen disorganization and degradation in the absence of inflammation (Khan et al., 2000). Tendinopathies have been reported to be the most frequent overuse injury in adolescent athletes (Mersmann et al., 2017), although they affect people of all ages and are common in both athletes and nonathletes.

Tendinopathies make up 30% of all musculoskeletal injuries (Andarawis-Puri et al., 2015) and are the most prevalent tendon disorder (Peters et al., 2016). This pathology in the lateral elbow is estimated to affect between 1.3% and 10.4% of adults each year (Thiese et al., 2016), with physician-documented recurrence in approximately 8.5% of cases.

However, because these statistics only include clinical diagnoses and are limited to those individuals who sought medical attention, they may underestimate the true value (Sanders et al., 2015).

They also do not account for patients diagnosed with medial epicondyle tendinopathy, which has an incidence that is approximately 10-25% that of lateral epicondyle tendinopathy (Mishra et al. 2014). The affected tendon in lateral elbow tendinopathy is the carpi radialis brevis muscle in the vast majority of cases, approximately 90% (Aben et al., 2017).

1.1.1. Who does elbow tendinopathy affect?

The prevalence in this pathology increases with age due to the degenerative effects of aging on tendons, with peak incidence between ages 35 and 55 (Aben et al., 2017); however, it is also diagnosed in younger athletes, and its prevalence in this group is increasing (Maffulli et al., 2003). The rehabilitation process for tendinopathy is slow and time-consuming, and can have significant effects on an athlete's sports career, quality of life, physical and psycho-social well-being (Peters et al. 2016). In athletics, individuals who practice sports that require overhead throwing or repeated forearm pronation and wrist flexion, such as baseball and swimming, represent a group with elevated risk, especially when those movements are combined with a tight handgrip, as in tennis and golf. This has led to the colloquial terms "tennis elbow" and "golfer's elbow" to describe tendinopathy of the tendons attaching to the lateral and medial epicondyles, respectively (Mishra et al., 2014 and Sanders et al., 2015).

Although this pathology often manifests in the dominant arm, it is also possible to occur in the non-dominant arm or bilaterally, even in regions of the tendon in which the load does not reach its maximum (Jost et al., 2005; Sanders et al., 2015). This observation suggests that other mechanisms are involved in the development of this pathology beyond the actual load to which the tendon is subjected.

1.1.2. Societal costs of elbow tendinopathy

Elbow tendinopathy has a substantial economic impact as a direct result of healthcare costs, worker absenteeism and reduced productivity (Aben et al., 2017). The degree of severity of this pathology is variable, in some cases being resolved with non-invasive interventions such as rest, bracing and administration of NSAIDs, and in other cases requiring open surgery. Approximately 12.2% of patients with recurrent lateral elbow tendinopathy went on to require surgical intervention.

It is therefore important to recognize the treatment costs associated with elbow tendinopathy in addition to its high incidence. An estimated 16% of patients diagnosed with lateral epicondyle report having work restrictions as a result of their condition, and 4% reported missing work (Sanders et al., 2015), and up to 5% of patients diagnosed with lateral or medial elbow tendon pathology are reported to go on long-term medical leave as a result of the disorder (Thiese et al., 2016).

1.1.3. What causes tendinopathy?

Tendons generate a mechanical response to loads, and being subjected to these loads is fundamental for regulating their physiological functions. However, excessive loads can lead to tendon injury, whether by an acute traumatic process of due to a chronic degenerative process resulting from the tendon's catabolic processes outweighing the anabolic processes, leading to degradation of the extracellular matrix (ECM) (Andarawis-Puri et al., 2015; Wang et al., 2012). However, as stated previously, the fact that tendinopathy can occur in the nondominant arm or in areas of the tendon that do not reach peak loading, and the fact that different patients' tendons respond differently to the same external load, has led to questions about other factors involved in the pathology.

In recent years, numerous studies have been carried out which analyze the relevance of other factors correlated with tendinopathy, including plasma glucose levels, cytokines, and genetic factors (Longo et al., 2009, Andersson et al., 2011 and Collins et al., 2009).

1.2. Justification

To date, several studies have described the contribution of genetic factors to the presence of Achilles and patellar tendinopathies (Mokone et al., 2006), but studies involving the upper limbs are scarce, with just a few studying genetic factors in rotator cuff tendinopathy

and, according to a literature search, only one study on elbow tendinopathy (Altinisik et al., 2015). This is one of the reasons the present study sought to analyze the presence of different genetic markers, as well as other known risk factors, in elbow tendinopathy.

Such knowledge could open new avenues to stratify the general population and athletes in particular according to their level of risk of suffering a tendon injury. Although clinicians must evaluate susceptibility on a case-by-case basis due to the multifactorial nature of tendinopathies, using potential genetic predisposition as another tool to be used in combination with family history, personal history of other tendon injuries, and level of participation in high-risk sports (Collins et al., 2015), a more thorough understanding of the role of certain genetic factors in this pathology could be a helpful tool in establishing preventive measures and developing individualized protocols in both treatment and training to minimize risk of this type of injury.

2. Background

2.1. Characteristics of the elbow

The elbow is a deceptively complex joint, with a unique anatomy that allows flexion-extension, supination-pronation and a small degree of rotation. It contains several static and dynamic stabilizers which serve as valgus and varus force restraints. The elbow is subject to very high valgus and varus forces compared to other joints,

especially in throwing athletes. Other stabilizers serve as restraints against axial and shear forces.

Tendon injury due to forceful eccentric contractions are more likely in muscles which span two joints, as is the case with the elbow, because they are rarely used at full physiological capacity and therefore fail at excessive loads outside of their usual range of function (Hsu et al., 2012). The elbow is also a very constrained joint, and as a result has some of the thinnest hyaline cartilage in the body (Osbahr et al., 2010).

2.1.1. Anatomy of the elbow

The elbow is a complex modified hinge joint (trocho-gynglymoid) with both rotator and hinge components.

2.1.1.1. Musculoskeletal

The elbow joint is made up of three articulations between the long bones of the arm: the ulnohumeral (between humerus and ulna), radiocapitellar (between humerus and radius), and proximal radioulnar (between radius and ulna) joints (Wells & Ablove, 2008). All three articulations are enclosed circumferentially by the joint capsule (Hsu et al., 2012).

The ulnohumeral or ulnotrochlear joint forms one part of the hinge component of the elbow joint and is formed by the trochlea of the distal humerus and the trochlear notch of the proximal ulna. The second part of the hinge is formed by the radiocapitellar joint, where the radial head meets the capitellum of the distal humerus (Chen et al., 2003; Wells & Ablove, 2008). Rotation occurs primarily through the radioulnar joint (Chen et al., 2003).

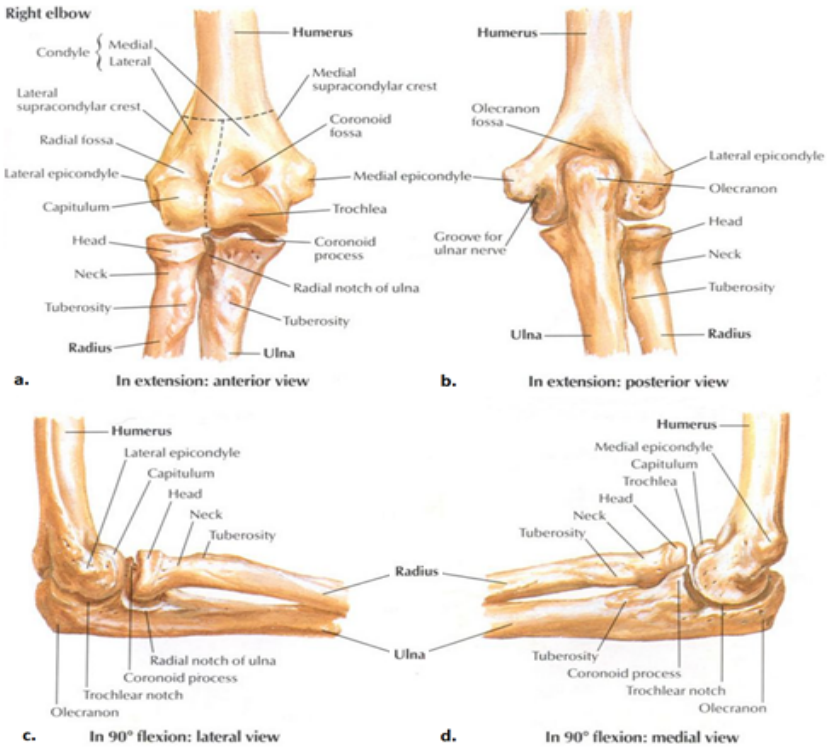

Figure 1. Skeletal anatomy of the right elbow joint, a) in extension, anterior; b) in extension, posterior; c) in 90º flexion, lateral; d) in 90º flexion, medial. Taken from Netter, 2014.

The most important ligaments for stabilizing the elbow joint are found in two ligamentous complexes: the medial collateral ligament (MCL) complex and the lateral collateral ligament (LCL) complex. The MCL consists of an anterior bundle, posterior bundle and a variable oblique-transverse bundle, and the LCL consists of the radial collateral ligament, lateral ulnar collateral ligament, annular ligament and occasionally an accessory collateral ligament (Hsu et al., 2012; Cain Jr. et al., 2003; Wells & Ablove, 2008).

Muscles which are proximal to the elbow joint include the elbow flexors and extensors: brachialis, biceps brachii and brachioradialis (flexors), and the triceps brachii (extensor), with their respective tendons. Distal to the elbow joint are the wrist flexor, extensor and supinator muscles.

The muscles which attach to the medial epicondyle of the humerus via the common flexor tendon are: flexor carpi radialis, flexor carpi ulnaris, flexor digitorum superficialis, pronator teres, and palmaris longus.

The common extensor tendon attaches to the lateral epicondyle of the humerus and is the origin of the supinator and some of the extensor muscles, including extensor carpi radialis brevis, extensor digitorum, extensor digiti minimi, extensor carpi ulnaris, the anconeus, and the supinator (Konin et al., 2013).

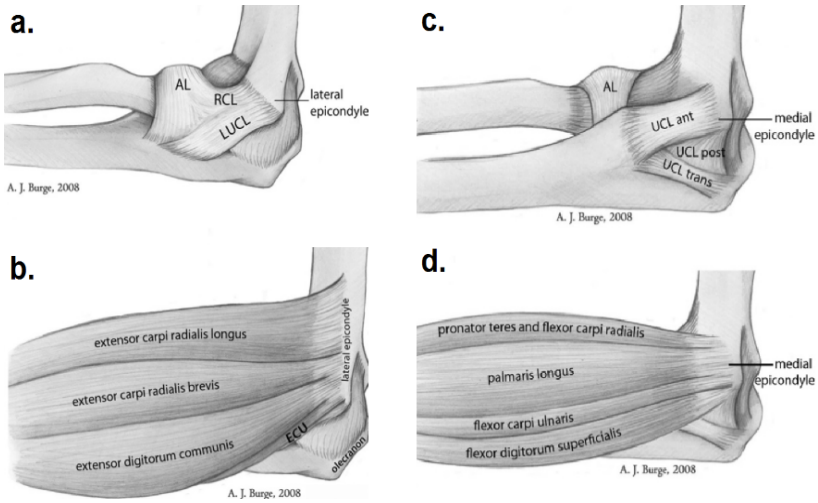

Figure 2. Normal structures of the (a-b) lateral elbow, and (c-d) medial elbow. AL = annular ligament, RCL = radial collateral ligament, LUCL = lateral ulnar collateral ligament, ECU = extensor carpi ulnaris, UCL = ulnar collateral ligament. Taken from Konin et al., 2013.

2.1.1.2. Vascularization

The elbow joint is supplied with blood by the brachial, deep brachial and ulnar collateral arteries which branch off of the brachial artery. The brachial artery runs anterior to the humerus and branches medially to form the ulnar collateral arteries, while the deep brachial

artery runs posterolaterally to the humerus before wrapping around anteriorly at the junction between the humerus and radius. These arteries are supplied by the subclavian artery by way of the axillary artery.

Anatomy of the veins at the elbow joint is similar to that of the arteries, with blood from the hand and forearm traveling through the median cubital vein, which is a branch from the basilica vein, the brachial vein and the cephalic vein, all anterior to the elbow joint. The brachial and basilica veins merge to form the axillary vein, which later merges with the cephalic vein to form the subclavian vein (Konin et al., 2013).

2.1.1.3. Innervation

Sensory and motor function at the elbow is supplied primarily by the median nerve, ulnar nerve, radial nerve and the medial brachial and antebrachial cutaneous nerves.

The median nerve runs anterior to the elbow, medial and parallel to the brachial artery, over the brachialis muscle and then penetrates between the two heads of the pronator teres muscle before passing under the flexor digitorum profundus muscle. It originates in spinal nerves C5-C8 and T1 by way of the lateral and medial cords of the brachial plexus, and directly innervates the pronator teres, flexor digitorum superficialis, palmaris longus, and flexor carpi radialis

muscles of the elbow joint. The anterior interosseous and palmar cutaneous nerves branch off of the median nerve inferior to the elbow joint. Sensory function from the median nerve is limited to the hand.

The ulnar nerve runs through the intermuscular septum at mid-arm, approximately 8 cm proximal to the medial epicondyle of the humerus. It passes into the cubital tunnel, running posterior to the medial epicondyle, before entering the anterior compartment by passing between the two heads of the flexor carpiulnaris muscle. It originates from spinal nerves C8 and T1 via the medial cord of the brachial plexus. It innervates the flexor carpi ulnaris and flexor digitorum profundus muscles of the elbow joint. Like the median nerve, sensory function of the ulnar nerve is limited to the hand.

The radial nerve emerges from the groove between the medial and lateral heads of the triceps and penetrates the lateral intermuscular septum approximately 8-12 cm proximal to the lateral epicondyle. It passes between the brachialis and brachioradialis and enters the radial tunnel just proximal to the capitellum of the humerus. It continues as the posterior interosseous nerve. It originates from spinal nerves C5-C8 and T1 via the posterior cord of the brachial plexus. It innervates the triceps brachii, anconeus, brachioradialis, extensor carpi radialis, and the supinator muscles. The posterior interosseous nerve then innervates the extensor digitorum, extensor digiti minimi, and extensor

carpi ulnaris muscles of the elbow. It is the primary nerve providing cutaneous sensory function to the posterior surface of the elbow.

The medial brachial and antebrachial cutaneous nerves provide most of the cutaneous sensory function to the anterior face of the elbow. They originate from C8 and T1 via the medial cord of the brachial plexus. The medial antebrachial cutaneous nerve runs medial to the axillary artery parallel to the humerus and continues down the ulnar side of the forearm medial to the brachial artery before branching into the volar branch and ulnar branch, which in turn distribute filaments to the skin.

2.1.2. Biomechanics of the elbow

The hinge component of the elbow joint has a flexion-extension range of motion from 0° (full extension) to 150° (full flexion) (Chen et al., 2003), although the degree of extension may be as high as -10° in some lax individuals (Taboadela, 2007). The distal humerus has two fossae which allow maximal flexion and extension of the elbow joint (Hsu et al., 2012). As mentioned previously, rotation occurs through the proximal radial ulnar joint, allowing supination and pronation of the wrist (both 85°) (Chen et al., 2003).

Both static and dynamic stabilizers, in the form of bony and soft tissues, play a role in the biomechanics of the elbow joint. During

flexion-extension, the interlocking bony anatomy of the olecranon process of the ulna with the olecranon fossa of the humerus provides stability at less than 20° extension and greater than 120°flexion. In the arc of movement between these points, most of the joint stability is provided by the ligaments, with the majority of stress falling on the MCL complex, specifically the anterior bundle. The anterior bundle of the MCL complex is the primary restraint of valgus forces, along with stability provided by the bony anatomy described above and dynamic muscle forces from the flexor carpi ulnaris and flexor digitorum superficialis. Valgus stress on the elbow generates tensile loads on the medial portion and compressive loads on the lateral and posterior portions of the elbow joint (Hsu et al., 2012).

In the case of an overhead throwing motion or axial load in weight-bearing sports, the lateral portion and the radiocapitellar joint absorb a significant portion of the axial and shear stress. These forces may lead to tendinopathy of the flexor-pronator musculature, namely the common flexor tendon at the medial epicondyle (“golfer’s elbow”) or the extensor carpi radialis on the lateral epicondyle (“tennis elbow”) (Hsu et al., 2012).

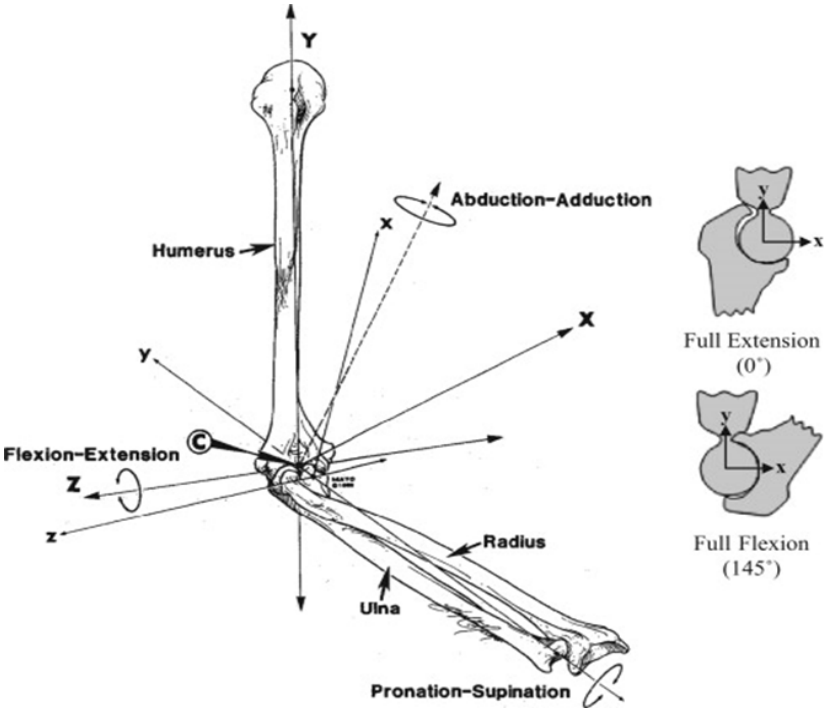

Figure 3. Elbow biomechanics of flexion-extension, abduction-adduction, and pronation-supination along xyz axes. Taken from Kincaid & An, 2013.

Anatomical studies have shown that the insertion site of the extensor carpi radialis brevis merges imperceptibly with the insertion site of the lateral collateral ligament, which in turn fuses with the annular ligament of the radioulnar joint. This anatomy suggests that considerable load sharing takes place between these structures (Benjamin et al., 2006).

2.2. Characteristics of tendons

Tendons are dense, fibrous structures of connective tissue that form the connection between muscle and bone, whose main function is to transfer the energy generated by the muscle to the joints in order to produce joint movement, while also helping to stabilize the movement (Thorpe et al., 2013; Connizzo et al., 2013; Franchi et al., 2007). As with other structures in the body, there is a close structural/functional relationship in the tendon (Zhang et al., 2005); consequently, the shape of the tendon varies according to the demands placed on the muscle-tendon complex, including flat tendons, cylindrical tendons, long and narrow tendons, short and thick tendons, and other forms (Franchi et al., 2007). However, all tendons have an internal hierarchy which gives them their unique characteristics.

2.2.1. Tendon composition

Tendons are formed by tendon fibroblast cells and highly organized collagen fibers in the extra cellular matrix (ECM), grouped into fascicles which are connected to each other by sheaths of connective tissue (Kannus 2000; Yoon & Halper, 2005). Under normal conditions, the dry mass of the tendon makes up approximately 30% of its total mass (Juneja & Veillette, 2013). Structurally, then, we can say that the tendon is made up of a set of cells situated in an ECM, whose principal component is collagen.

2.2.1.1. Cells of the tendon

Approximately 90-95% of the cells observed in tendons are fibroblasts (tenoblasts), although endothelial cells and chondrocytes can also be found (Franchi et al., 2007). At birth, the majority of cells found in tendons are tenoblasts, which later mature into tenocytes, which have a more elongated shape and less metabolic activity than tenoblasts (Kannus 2000; Franchi et al., 2007).

Tenoblasts are responsible for synthesizing the precursor peptides for collagen and other components of the ECM; as such, they play an important role in tendon repair and maintenance, maintaining homeostasis and adapting to environmental changes (Kjaer et al., 2009). Tenocytes produce energy through the Krebs cycle, anaerobic glycolysis and the pentose phosphate pathway, although with age, energy production becomes primarily anaerobic (Kannus 2000).

Figure 4. Illustration of tendon development, transcriptional factors in red and other molecular markers in green. SCX = scleraxis; MKX = mohawk; EGR = early growth response; TDSC = tendon-derived stem cells; TNMD = tenomodulin; TNC = tenascin C; TBHS4 = thrombospondin-4. Taken from Lu et al., 2016.

2.2.1.2. Tendon extracellular matrix

Type I collagen is the principal component of the tendon ECM, comprising approximately 60-85% of the dry mass of a normal tendon (Tresoldi et al., 2013). Collagen is explained in greater detail in section 2.2.2, “Collagen”.

The small remainder of the non-collagen ECM is periodically intercalated in each level of collagen hierarchy and is primarily composed of glycoproteins, the majority of which are proteoglycans; cartilage oligomeric matrix protein, tenascin-C, lubricin and fibronectin can also be found, as well as variable proportions of elastin.

Proteoglycans (PGs) are composed of a protein nucleus bonded to one or more glycosaminoglycan (GAG) chains via covalent bonding (Yoon & Halper, 2005). These GAGs vary according to the location of the tendon and the type of tension it supports, yielding varying concentrations of hyaluronic acid, chondroitin and dermatan sulfate, keratin sulfate and heparin (Kannus, 2000; Juneja & Veillette, 2013). The majority of PGs found in the tendon are small leucine-rich proteoglycans (SLRPs), although larger PGs can also be found, such as agrecan and versican.

Decorin is the most abundant of the SLRPs, making up approximately 80% of the total PG content in the tendon; however, smaller quantities of biglycan, fibromodulin and lumican can also be

found (Thorpe et al., 2013). Although the different PGs play different roles depending on their type, in general they have an important role in the fusion of collagen fibers as well as in their alignment (Kjaer 2004). They also modulate cell growth and the maturation and differentiation of tissues. They may also act as a biological filter, modulating the activity of growth factors and regulating collagen fibrillogenesis (Yoon & Halper, 2005).

Tenascin-C (TNC) is an elastic protein that can be stretched to several times its resting length. This ability to vary its length is the origin of its elastic properties. TNC also contributes to the orientation and alignment of collagen fibers (Järvinen et al., 2003). The expression of this protein is regulated by mechanical stress, which is why in adult tendons it is predominantly expressed in regions that transmit high levels of mechanical force, such as the myotendinous and osteotendinous junctions (Juneja & Veillette, 2013).

Cartilage oligomeric matrix protein (COMP) interacts with collagen, the cells and other ECM proteins in the tendon. Its main function is to collaborate in fibrillogenesis.

2.2.2. Collagen

Collagen is the most abundant protein in the tendon's ECM, and its function is related to the formation of fibril and microfibril

substances of the ECM. It also plays an important role in determining the specific properties of each tissue (Tresoldi et al., 2013). Approximately 63% of a collagen molecule is made up of three amino acids: glycine (33%), proline (15%) and hydroxyproline (15%).

2.2.2.1. Collagen structure and organization

Collagen has a hierarchical structure from lesser to greater complexity. The simplest structural unit of this hierarchy is the soluble tropocollagen molecule, which is formed in tenoblast cells as procollagen. The tropocollagen molecules each consist of a left-handed helix. Three of these chains coil to form a right-handed superhelix, with two identical α-1 chains and a slightly different α-2 chain being connected by hydrogen bonds via glycine residues, forming an insoluble rope-like structure known as a microfibril (Connizzo et al., 2013; Sharma & Maffulli, 2006), as illustrated in Figure 5.

These microfibrils, in turn, crosslink with each other in a staggered alignment to form fibrils, the alignment of which is heterogeneous but locally aligned mainly with the direction of the load, enthesis or bone insertion (Thompson, 2013).

Collagen fibrils group together to form fibers known as primary bundles. Primary bundles combine to form secondary bundles (fascicles), which in turn combine to form tertiary bundles which make up the tendon. Beginning at the level of the secondary bundle, the characteristic "crimp" of the collagen structure can be observed (Killian et al., 2012). As stated earlier, at each hierarchical level, non-collagen components of the ECM are intercalated with the collagen, and tenoblasts and tenocytes can be found periodically between fibrils.

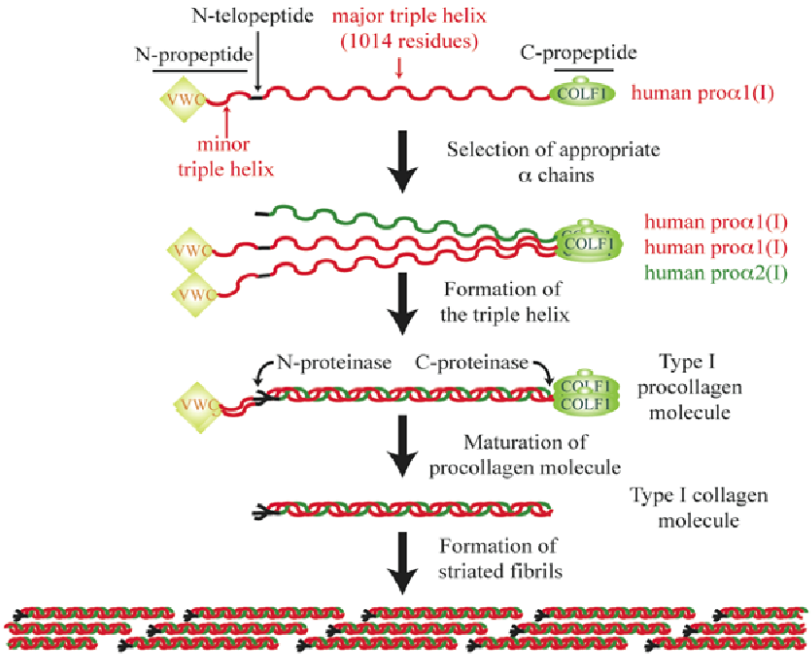

Figure 5. Illustration of steps from pro-collagen fibrillar chains to striated collagen fibrils in Type I collagen. Taken from Exposito et al., 2010.

2.2.2.2. Synthesis and degradation

There are multiple types of collagen and each plays a different role in the tendon, requiring an effective process for synthesis and degradation of collagen. Synthesis begins intracellularly in tenoblast cells, with the transcription of mRNA for each alpha chain and subsequent translation of the polypeptide chains on the rough endoplasmic reticulum, forming procollagen molecules, which are secreted and transformed extracellularly into collagen and grouped into increasingly complex structures as described above (Kjaer, 2004).

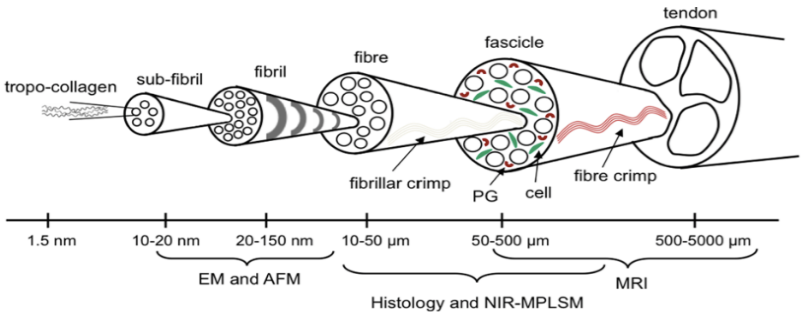

Figure 6. Hierarchical organization of collagen in the tendon. Taken from Thompson, 2013.

Collagen synthesis is influenced by several growth factors which regulate its genetic activation, such as insulin-like growth factor, transforming growth factor beta, interleukin, fibroblast growth factor, prostaglandins, and vasoactive endothelial growth factor. Load on the tendon is a fundamental factor in the activation of collagen synthesis. Adequate degradation is also required to maintain equilibrium in

collagen exchange, which is believed to be mediated primarily by matrix metalloproteinases (MMP) and mitogen activated protein kinases (MAPK) in the extracellular environment (Kjaer, 2004).

2.2.2.3. Collagen types

Type I collagen is the most abundant type of collagen in tendons, varying from 96-98% of the total collagen (Juneja & Veillette, 2013) and playing an important role in the mechanical properties of tendons (Killian et al., 2012). Variable amounts of other collagen types can also be found, including types III, V, XI, XII and XIV (Tresoldi et al., 2013). Type III collagen plays a key role in regulations of fibrillogenesis and extensibility of the tendon. Types V and XI are associated with types I and II, respectively, determining their quantity and quality (Wenstrup et al., 2011), while types XII and XIV are fibril-associated collagens with interrupted triple helices.

2.2.3. Structural organization of the tendon

Collagen fibers are woven into fiber bundles by the endotendon. This layer forms a reticular web of connective tissue through which blood vessels, lymphatic vessels and nerves run (Sharma & Maffulli, 2006). This layer is also related with hydration of proteoglycans between the endotendon and the fascicles (Franchi et al., 2007).

Figure 7. Collagen fibers in a healthy rat patellar tendon. Collagen fibers are organized in a parallel alignment in healthy tendons. Taken from Franchi et al., 2007.

Above the endotendon is a delicate layer of lax connective tissue known as the epitendon (Tresoldi et al., 2013). This layer forms a relatively dense fibrillar web of collagen where mostly longitudinal fibers are found, although it is possible to find transverse and oblique fibers as well (Kannus, 2000). The surface of the epitendon is covered by the paratendon, which is an areolar lax connective tissue made of type I and III collagen, as well as a small number of elastic fibrils. Lining the interior of this layer are synovial cells (Sharma & Maffulli, 2006). This peritendinous tissue acts as an elastic layer, allowing the movement of the tendon against nearby tissues (Franchi et al., 2007).

Apart from these common elements, different tendons show a series of structures that surround the tendon and which facilitate its gliding and lubrication according to the shape and function of each

tendon. Synovial layers can be found in areas of greater mechanical stress, acting as fulcrums and providing the tendon with a greater mechanical advantage (Franchi et al., 2007; Kannus, 2000; Sharma & Maffulli, 2006).

2.2.4. Blood supply to the tendon

At maturity, tendons are largely avascular. During development, they possess a profuse network of capillaries; however, vascularization is compromised in such a way that the vascularized area of a mature tendon is just 1-2% (Kjaer, 2004; Fenwick et al., 2002).

Tendons receive their blood supply from three principal sources: the intrinsic systems of the myotendinous junction (MTJ), those originating from the osteotendinous junction (OTJ), and the extrinsic system formed by blood vessels originating from adjacent connective tissues, such as the paratendon, mesotendon or synovial sheaths (Fenwick et al., 2002). The ratio of blood supplied by each system varies according to the tendon's function.

In the MTJ, the arteries and arterioles originate in the perimysium, continuing through the tendon's fascicles (Kjaer, 2004); however, this system only provides blood to the first one-third of the tendon (Sharma & Maffulli, 2005). In the case of the OTJ, blood

vessels do not pass directly from bone to tendon but are anastomosed with those of the periosteum, forming an indirect link with osseous circulation.

The blood supply originating in the extrinsic system varies according to whether or not the tendon is surrounded by a synovial sheath to avoid friction. If it has, branches from the large vessels pass through the mesotendon, arriving to the visceral layer of the synovial sheath, where a plexus is formed which irrigates the superficial layers of the tendon. Some of these blood vessels penetrate the endotendon, forming connections between the peritendon and intratendon. In tendons without the synovial sheath, extrinsic irrigation originates in the paratendon. In this region, blood vessels run transversely, branching out to form a complex vascular network. Some arterial branches of the paratendon cross the epitendon until they reach the endotendon, where they form anastomose that provides irrigation to the internal area of the tendon (Sharma & Maffulli, 2005).

2.2.5. Innervation of the tendon

The nerves of tendons run through the trunks of cutaneous, muscular and peritendinous nerves. At the MTJ, fibers in the tendon form nervous plexus at the level of the paratendon. Some of these plexus originate in branches that come from the endotendon; however,

the majority of nervous fibers do not originate in the main body of the tendon but in the superficial nerve endings (Sharma & Maffulli, 2005).

Both myelinated and amyelinated fibers can be found in tendons. The myelinated fibers constitute the specialized mechanoreceptors for detecting changes in pressure or tension. These mechanoreceptors are Golgi organs, and are more numerous in the MJT. The amyelinated fibers, on the other hand, correspond to the nociceptors which sense and transmit pain (Sharma & Maffulli, 2005).

2.2.6. Biomechanics of the tendon

Tendons are structures responsible for transferring force from muscles to bones. Apart from this, they have the ability to absorb the energy produced and relax this elastic energy, thereby limiting damage to the muscle (Connizzo et al., 2013; Seynnes et al., 2015).

Because of this, the tendon has unique abilities of rigidity and flexibility that guarantee adequate functioning in which mechanical efficiency and efficacy dominate (Seynnes et al., 2015). This ability is due to the properties conferred by the structure of the tendon. Therefore, we can consider them viscoelastic tissues with nonlinear, anisotropic formation which exhibit great mechanical force, good flexibility and elasticity (Connizzo et al., 2013; Sharma & Maffulli, 2005; Seynnes et al., 2015).

The nonlinear behavior of the tendon is reflected in an effort-deformation curve divided into four zones. In the first zone, a nonlinear baseline region is observed, characterized by presenting little rigidity when stretching is less than 2%. In this stage, an unfolding of collagen fibers is produced which, as mentioned earlier, tend to form a spiral or crimp shape (Connizzo et al., 2013; Thompson, 2013; Herchenhan et al., 2012).

Once this unfolding has taken place, a stretching is produced at the molecular level with extension of the fibrils, resulting in deformation of the tendon if the tension is excessive. This deformation is produced in part due to an extension of the fibers that occurs in only 40% of the tissues while the rest of the deformation is due to a sliding of these fibers (Herchenhan et al., 2012).

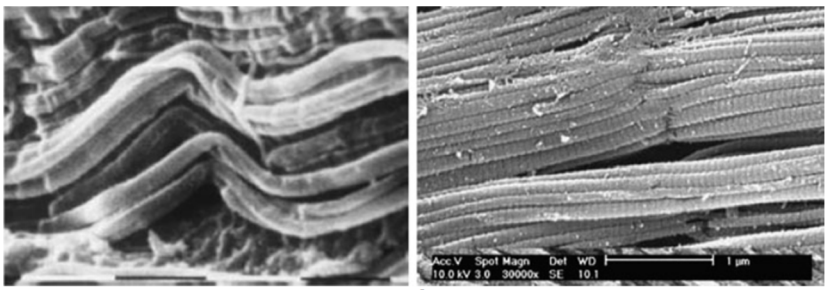

Figure 8. Collagen fibers (a) before and (b) after the tendon is subjected to tension. The "unfolding" of the characteristic crimp structure of the collagen can be observed. Taken from Thompson, 2013.

It is the linear region where the tissues reach the upper limit of stretching and around 4% of stretching is produced. From this point, microscopic breakages can be observed, possibly becoming macroscopic if the stress continues to increase above 8-10%, leading to breakage of the tendon (Wang et al., 2012).

The viscoelastic component of the tendon gives it its ability to adapt to the type of load to which is it subjected, such that tendons are more deformable with low loads since they absorb more energy, leading to lower mechanical efficiency. Conversely, when subjected to large loads, tendons are less deformable and it is this rigidity that allows them to be more effective when transferring large loads to the bone (Connizzo et al., 2013; Wang et al., 2012).

2.2.7. Physiological response to load

Tendons generate a mechanical response to loads, and being subjected to these loads is fundamental for regulating their physiological functions. However, excessive loads can lead to tendon injury, whether by an acute traumatic process of due to a chronic degenerative process resulting from the tendon's catabolic processes outweighing the anabolic processes, leading to degradation of the ECM (Andarawis-Puri et al., 2015; Wang et al., 2012). When tendons are subjected to a uniaxial tension in the direction of the predominant alignment of the collagen fibers, matrix components contribute to

maintaining nonlinear, anisotropic and viscoelastic characteristics of the tendon (Connizzo et al., 2013).

The physiological changes produced by adequate loading the tendon are many, with adequate load differing depending on the characteristics of the tendon and the subject themselves. Generally speaking, tendons operate under normal conditions between 30% and 40% of their ultimate tensile strength (UTS); however, differences can be found from one tendon to the next. For example, the Achilles tendon can operate in a range of 50-100% UTS, while the supraspinous tends to work in the range of 25-30% UTS (Thornton & Hart, 2011).

2.2.7.1. Physiological effects of exercise on the tendon

Healthy, repetitive loading, as in the case of exercise, can promote tendon remodeling, leading to long-term improvements in structure and function. The process of tendon remodeling involves both the synthesis and degradation of collagen, which begins immediately after exercise and yields a net synthesis (Andarawis-Puri et al., 2015; Wang et al., 2012; Magnusson et al., 2010).

The adaptive response to exercise leads to an increase in synthesis and turnover of matrix proteins, especially collagen. This increase in the formation of collagen occurs during both acute and chronic loading (Andarawis-Puri et al., 2015; Kjaer et al., 2009; Wang et al., 2012). However, differences have been found in the increase in

collagen synthesis in men vs. women, with a lower post-exercise response in women. This factor could be influenced by the presence of estrogens (Kjaer et al., 2009).

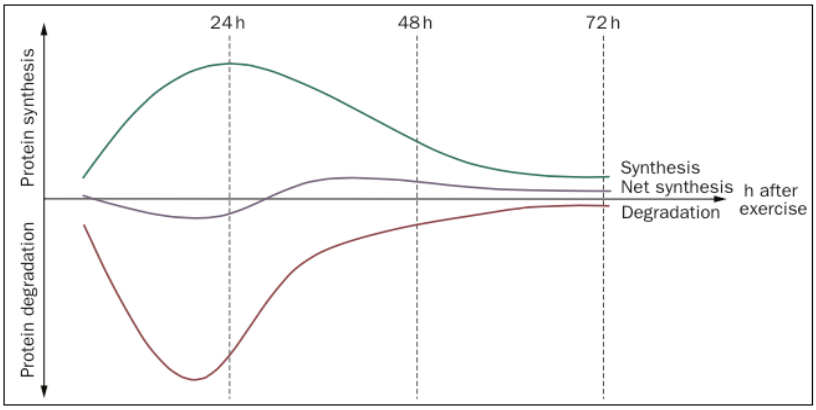

Figure 9. Diagram of collagen synthesis and degradation in tendons in the 72-hour period post-exercise. Taken from Magnusson et al., 2010.

With regard to the changes to the PG profile in response to loading, there are differences depending on the variety of PG and the type of load. Generally speaking, there is an increase in levels of decorin and a decrease in levels of agrecan (Yoon & Halper, 2005).

In different studies in animal models, it has been observed that a physiological exercise protocol promotes cell proliferation, especially progenitor cells from tendon stem cells (2), as well as an increase in the expression of genes associated with tenocytes without affecting the expression of adipocytes, chondrocytes, or osteocytes (Andarawis-Puri et al., 2015; Wang et al., 2012). Additionally,

adequate loading is necessary to improve repair in injured tendons, evidenced by the reduction in adhesions in flexor tendon injuries (Wang et al., 2012).

These changes are observed differently in acute and chronic exercise. Regarding acute effects, during the exercise, a series of changes is produced which involves multiple signaling pathways and mediators, including changes in intracellular calcium via the activation of calcium channels, increasing levels of calcium in circulation and allowing release of ATP. There is also a change in the organization of cytoplasmic filaments (especially actin) and protein expression is modified by secretion of MMPs (Lavagnino et al., 2015).

The most important changes for understanding the acute implications of exercise on the tendon are: increase in synthesis and degradation of collagen with net synthesis after a period of 36-72 hours (Kjaer, 2004; Magnusson et al., 2010); increase in growth factors IGF-1, TGF-β, CTGF and VEGF, among others (Lavagnino et al., 2015); increase in MMPs (Andarawis-Puri et al., 2015; Neviaser et al., 2012); increase in cellular proliferation (Andarawis-Puri et al., 2015; Yoon & Halper, 2005; Cook & Purdam, 2012; Zhang & Wang, 2013); dose-response changes in tenascin-C (Magnusson et al., 2010; Couppé et al., 2008); increase in prostaglandins and interleukin-6 (Kjaer 2004; Lavagnino et al., 2015); and increase in blood flow (Andarawis-Puri et al., 2015; Neviaser et al., 2012; Cook & Purdam, 2012).

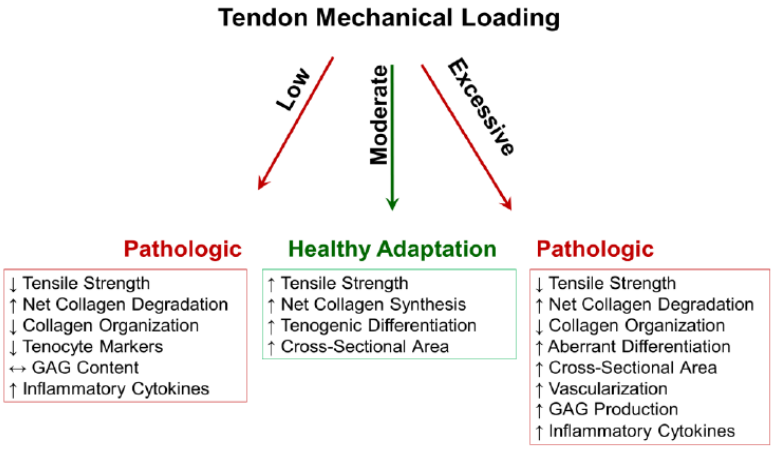

Figure 10. Pathways that may occur following low, moderate, and excessive loading of the tendon. Taken from Freedman et al., 2015.

Regarding chronic effects, as a consequence of an exercise protocol carried out over the course of several weeks or months, structural changes can be seen in the tendon with an increase in total number of PGs (Yoon & Halper, 2005) and tenocytes (Zhang & Wang, 2013). Furthermore, there is evidence of changes in the tendon's mechanical properties (Kjaer et al., 2009) and increase in rigidity and extensibility (Zhang & Wang, 2013; Couppé et al., 2008), thereby producing an increase in the transversal area of the tendon (Kjaer et al., 2009; Zhang & Wang, 2013; Couppé et al., 2008). Another fundamental adaptation that results from long-term training is an increase in collagen synthesis, caused by an increase in growth factors such as TGFβ-1 and IGF-1 (Heinemeier & Kjaer, 2011).

2.3. Tendon pathology and tendinopathy

The term tendinopathy describes clinical conditions in and around the tendon associated with pain accompanied by a mechanical, degenerative pathology resulting from overuse (Thornton & Hart, 2011). However, this pathology is not always associated with pain or overuse, even though imaging reveals structural disorganization of collagen in the tendon. The majority of tendinopathies occur at or near the osseous insertion of the tendon (Cook & Purdam, 2012).

A more extensive model of tendinopathy, as opposed to other tendon injuries, is the lack of inflammatory response, which explains the chronicity of the injuries as a result of failure to repair after being subjected to an excessive mechanic load. Currently, this process is considered to be something of a continuum of changes that can move from an acute, inflammatory response to non-pathologic stages or to more advanced stages with degenerative changes (Cook & Purdam, 2009).

This model of tendinopathy as a continuum process was proposed by Cook and Purdam in 2009 and is based on the existence of three distinct phases of pathology which are continuous and bidirectional, thereby establishing a continuum where each phase may overlap with the previous or subsequent one.

As such, it is possible to find a first pathological phase called reactive tendinopathy, characterized by a homogeneous proliferative non-inflammatory response. This phase is generally the result of an abrupt increase in load or a direct impact to the tendon. As a result, the tension and compression forces on the tendon increase sharply. For this reason, it is a short-term response. To counter the load, the tendon increases its thickness, which allows it to reduce stress, whether by increase in cross-sectional area or adaptation to the compression. This phase can generally be observed in young athletes who increase their training load, being reversible with an adjustment of the load (Cook & Purdam, 2009).

The second phase is given by a failure in reparation, called "tendon dysrepair" by the authors. This phase describes the inflammation attempt of the tendon, and is comparable to the first phase but with greater alterations in the ECM. It is most characteristic in young athletes subject to chronic loads or elderly subjects subject to moderate loads. This phase can be reversed with proper management of load and specific exercises (Cook & Purdam, 2009).

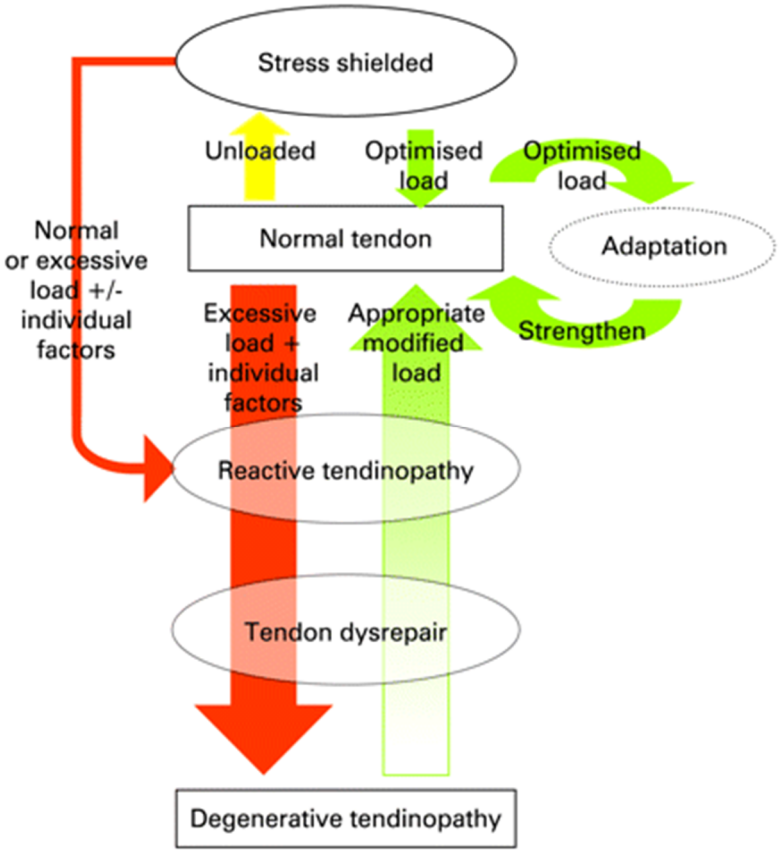

Figure 11. Continuum model of tendinopathy. Taken from Cook & Purdam, 2009.

The last phase is the degenerative phase, nearly irreversible, where the tendon suffers greater structural damage. It is characteristic in subjects to have suffered repeated episodes of tendinopathy, elderly subjects and elite athletes subject to intense, chronic loading. In this phase, the risk of breakage is higher (Cook & Purdam, 2009).

Genetic and other intrinsic factors influencing risk for elbow tendinopathy

2.3.1. Types of tendon pathologies

Due to the biological characteristics of the tendon, their regulation requires them to be subjected to physiological loading. However, when these loads are excessive, they can cause tendon injury, whether because of an acute, traumatic injury or via a chronic degenerative process due to the accumulation of microtraumas and altered cellular response (Lavagnino et al., 2015).

2.3.1.1. Acute tendinopathy

In this type of injury, a single load exceeds the maximum threshold that the tendon is capable of supporting, compromising its integrity. Generally, the response to an acute injury includes a process of inflammation, healing, remodeling and scarring over the injured tissue. This type of injury includes total or partial breakages; however, these breakages are normally preceded by degenerative changes and are not frequently observed in healthy tendons (Thornton & Hart, 2011).

2.3.1.2. Chronic tendinopathy

Chronic tendinopathies are a consequence of continuous overuse associated with painful, subacute chronic loss of function. In this pathology, the tendon is exposed to loads that exceed the

adaptation threshold or repetitive stimuli which exceed the repair threshold (Thornton & Hart, 2011).

2.3.1.3. Other tendon pathologies

There has been some confusion in the clinical community regarding terminology for tendon disorders. The term “tendinopathy” is, in fact, a relatively recent addition to the physiotherapy lexicon, having previously been labeled as “tendinitis”. It has been determined that most clinical presentations of painful overuse tendon conditions have a non-inflammatory pathology, and thus the term “tendinitis” is not appropriate in most cases since it implies that inflammation is involved in the mechanism of the pathology (Khan et al., 2002). Inflammatory tendinitis has also been reported (Dakin et al., 2015), although this is much less frequent in a typical clinical setting (Khan et al., 2002).

Tendon tears and ruptures can also occur as a result of acute overloading or laceration, and these injuries often present in chronically degenerated tendons, i.e., tendons suffering from tendinopathy (Thomopolous et al., 2015).

2.3.2. Pathological changes in tendinopathy

The typical pathological changes associated with tendinopathy include degeneration and disorganization of the collagen structure (Sharma & Maffulli, 2006; Cook & Purdam, 2012; de Giorgi et al., 2014), the result of a greater mRNA expression of collagen type I and III, which leads to an increase in the production of type III collagen (Magnusson et al., 2010; Cook & Purdam, 2012). Although there is also an increase in type I collagen, due to the alteration of collagen homeostasis, there is no net increase as the catabolic processes outweigh the anabolic processes. The increase in type III collagen is associated with the scarring process (Killian et al., 2012).

Although many authors report a decrease in the number of fibroblasts (Magnusson et al., 2010), according to various authors a cell proliferation is produced rather than tenocyte apoptosis (Andersson et al., 2011; Cook & Purdam, 2012), finding more rounded cells with a larger endoplasmic reticulum (Cook & Purdam, 2012). However, deformation and shortening of the tenocytes is observed.

Due to excessive load and poor adaptation to this load, a decrease in proliferation of tendon stem cells, favoring differentiation of these cells into non-tenocytes, such as adipocytes, chondrocytes and osteocytes (Zhang & Wang, 2013), thereby favoring the infiltration of fat and calcification of the tendon (de Giorgi et al., 2014). These

differentiation processes are mediated by an increase in certain genes, including PPARc, Sox9 and Runx2, as well as an increase in PGE2 (Zhang & Wang, 2013).

In relation to the components in the tendon, an increase in proteoglycans is observed, as well as an increase in certain GAGs (Magnusson et al., 2010; de Giorgi et al., 2014) such as chondroitin sulfate, agrecan and biglucan (Cook & Purdam, 2012). There is also an increase in fibromodulin and evidence of greater proportions of tenascin C and fibronectin (Magnusson et al., 2010).

There is an increase in some degenerative enzymes, such as ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) and MMPs (de Giorgi et al., 2014). On the other hand, a decrease is seen in the tissue inhibitor metalloproteinase TIMP3 (Magnusson et al., 2010). These changes are related to alterations in the structure of the tendon and can weaken the tendon's EMC (de Giorgi et al., 2014).

Although many authors note the absence of an inflammatory process per se, it is possible to observe a molecular "inflammation" at the local level, mostly mediated by the expression of MMPs and COX2 as well as PGE2 (Lavagnino et al., 2015). PGE2 is a mediator of pain and inflammation of tendons, reducing the proliferation of fibroblasts and collagen production (Wang et al., 2012).

Another controversial point is the significance of blood supply to the contribution/perpetuation of tendinopathy. While poor irrigation of the tendons has been associated as a cause of tendon pathologies, the presence of neovascularization and increase in intratendon blood flow has been observed in a large number of pathologies, including Achilles, patellar, epicondylar and rotator cuff tendinopathies (Andersson et al., 2011; Magnusson et al., 2010; Mousavizadeh et al., 2014). This pro-angiogenic response is mediated primarily by expression of VEGF (Magnusson et al., 2010; Mousavizadeh et al., 2014). Recently, the influence of angiopoietin 4 as another precursor to neovascularization has been studied (Mousavizadeh et al., 2014).

This angiogenesis can be accompanied by neurogenesis, that is, by the formation of new nerves with an increase in the level of substance P, calcitonin and other substances related with pain (Magnusson et al., 2010).

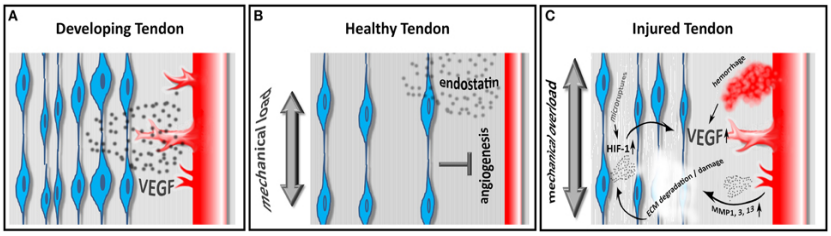

Figure 12. Vascularization of the tendon at different stages. A) In the developing tendon, high production of VEGF results in angiogenic response. B) In the healthy adult tendon, tenocytes produce the antiangiogenic factor endostatin in response to moderate physiological load. C) In the injured tendon, excessive loading causes tenocytes to produce HIF-1, inducing the expression of VEGF and thus angiogenesis. Taken from Tempfer & Traweger, 2015.

Keeping the continuum model proposed by Cook and Purdam (2009) in mind, the changes produced will be different according to which phase the tendon is in. In the first characteristic phase of reactive tendinopathy, cellular changes are produced with an increase in fibroblasts and greater presence of PG, associated with an increase in water in the ECM. The integrity of the collagen is practically unchanged, although some degree of longitudinal separation can be observed (Cook & Purdam, 2009).

In the second phase, there is evidence of greater changes to the ECM, an increase in collagen fiber separation and disorganization of the ECM. There is also an increase in vascularization and neurogenesis (Cook & Purdam, 2009).

Finally, in the degenerative phase, cellular apoptosis is observed accompanied by trauma in tenocytes, disorganization of the ECM, increase in vascularization and neurogenesis. The tendon may present nodular areas where areas of degeneration are mixed with healthy areas (Cook & Purdam, 2009).

2.3.2. Elbow tendinopathy

Tendinopathies of the upper extremities have not been as heavily studied as those of the lower extremities, and elbow

tendinopathy in particular appears in the literature less often than more prevalent pathologies like rotator cuff tendinopathy. However, they are a relatively common condition among both athletes and non-athletes (Sanders et al., 2015), although amateur athletes are more likely to suffer from elbow tendinopathy than professionals (Mishra et al., 2014). Subjects over the age of 40 are most likely to suffer from this pathology (Mishra et al., 2014; Sanders et al., 2015).

Tendinopathy of the elbow occurs almost exclusively at the tendon attachments of the wrist flexor and extensor muscles, with the most reported pathology of biceps and triceps tendons being tendon rupture (Hsu et al., 2012). Tendinopathies of the common extensor tendon and common flexor tendon are prevalent enough to have received the colloquial names “tennis elbow” and “golfer’s elbow”, respectively. They are classified as insertional tendinopathies (Benjamin et al., 2006).

2.3.2.1. Lateral elbow tendinopathy, “tennis elbow”

Tennis elbow has long been known as lateral epicondylitis, despite current understanding that inflammatory processes are rarely involved in this condition, and tendinopathy is a more accurate (or at least less confusing) term (Benjamin et al., 2006). It is a degenerative condition of the common extensor tendon, which attaches many of the

wrist extensor-supinator muscles at the lateral epicondyle of the humerus (Sanders et al., 2015; Altinisk et al., 2015).

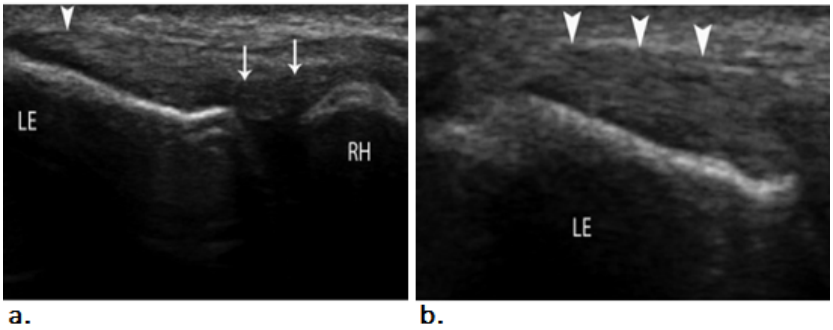

Figure 13 Ultrasound (US) of a normal common extensor tendon, (a) longitudinal and (b) short axis. Arrows in a = normal RCL, arrow heads in a and b = common extensor tendon. LE = lateral epicondyle, RH = radial head. Taken from Konin et al., 2013.

Despite the colloquial term, this pathology affects not only tennis players, but many people who participate in activities that involve repetitive wrist motion or a power grip (Altinisk et al., 2015), including throwing sports and even driving (Hsu et al., 2012). The true incidence of lateral elbow tendinopathy is not well-understood, but was previously estimated to affect 1-3% of adults each year, with a recent population-based study calculating an annual sex- and age-adjusted incidence of 3.4 per 1000. The incidence was found to be slightly higher in women (3.5 per 1000 vs. 3.3 per 1000), and highest in the 40-49 age group (7.8 per 1000 in males, 10.2 per 1000 in females) (Sanders et al., 2015).

There is no standardized protocol for treatment of lateral elbow tendinopathy, but the majority of patients are initially treated non-invasively. In the population-based study by Sanders et al. (2015), the most common treatment within the first year of diagnosis was symptom management with NSAIDs (82%), followed by bracing (77%) and physical therapy (35%). They reported a recurrence rate of 8.5% with a median recurrence time of 19.7 months; 12.2% of these patients (2% of the total sample) required surgery within two years of diagnosis. The authors reported that patients with no symptom resolution within 6 months may benefit from surgical intervention (Sanders et al., 2015).

Lateral elbow tendinopathy has been reported to manifest primarily in the right arm (63%), with 25% occurring in the left arm and 12% manifesting bilaterally (Sanders et al., 2015). Given that the general consensus is that 10% of the population is left-handed, we can infer that lateral elbow tendinopathy occurs more frequently in the dominant arm but may also occur on the non-dominant side (Sanders et al., 2015).

2.3.2.2. Medial elbow tendinopathy, “golfer’s elbow”

Medial elbow tendinopathy or golfer’s elbow is not as common as lateral tendinopathy, which is diagnosed approximately 4 to 10 times more often than the medial pathology. Like tennis elbow,

it has been given the misnomer medial epicondylitis, suggesting an inflammatory pathology when it is, in fact, a degenerative one.

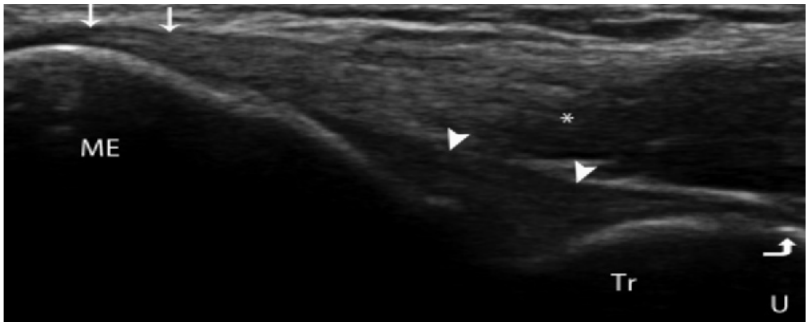

Figure 14. Ultrasound of a normal common flexor tendon (straight arrows) and UCL (arrow heads), longitudinal. ME = medial epicondyle, * = myotendinous junction, Tr = trochlea, U = ulna. The common flexor tendon is shorter and thicker than the common extensor tendon. Taken from Konin et al., 2013.

In agreement with the tendinopathy continuum model described previously, histologic data through different stages of golfer's elbow have shown a brief inflammatory response followed by degeneration, including microtearing, disorganization of collagen architecture, incomplete vascular response and angiofibroblastic degeneration, ultimately leading to structural failure (Mishra et al., 2014).

This pathology primarily affects the common flexor tendon, which attaches the wrist flexor-pronator musculature to the medial epicondyle of the humerus. Sports associated with medial elbow tendinopathy include golf, tennis, baseball, javelin, swimming, American football, rock climbing and archery. Repetitive bending and

straightening of the elbow has also been associated with tendinopathy (Mishra et al., 2014).

2.3.3. Diagnosis and clinical presentation of tendinopathy

Tendon pathology is not always painful, but clinical presentation tends to be associated with pain (Rio et al., 2014; Dean et al., 2015). However, it is possible for subjects with no history of pain to suffer a spontaneous tendon breakage due to sufficient structural disorganization to alter the integrity of the tendon (Kannus & Józsa, 1991). It could be said, then, that there is a poor association between the severity of symptoms and the extent of structural changes (Rio et al., 2014; Dean et al., 2015), with little or no correlation being established between tissue damage observed via imaging techniques and clinical presentation (Rio et al., 2014).

Tendinopathy is clinically characterized by:

- Localized pain when subjected to loading
- Tenderness upon palpation
- Change in function
- On/off nature of symptoms associated with loading
- Absence of pain at rest
- Decrease in symptoms at the beginning of activity, with possible peaks in pain depending on the action performed.

The evaluation of these signs and symptoms is done through an initial interview as well as a clinical exam that may include different pain and function questionnaires, specific tests and evaluation of range of mobility. It is possible to evaluate the magnitude of structural changes through microscopic examination of a tissue biopsy (Rio et al., 2014; Dean et al., 2015) or using imaging techniques such as magnetic resonance or ultrasound (Rio et al., 2014). No significant differences are observed regarding the accuracy of both techniques, and either can be employed to evaluate different tendon pathologies.

However, ultrasound has the advantage of being a noninvasive technique that is relatively inexpensive and fast, providing the technician or specialist with a real-time, high-resolution dynamic image that allows them to assess the possible changes produced in the tendon (McAuliffe et al., 2016).

2.3.4. Risk factors associated with tendinopathy

As stated earlier, under normal conditions, tendons respond by adapting to the load imposed on them; however, each subject has a unique response capacity depending on their individual characteristics. Several characteristics predispose a subject to greater or lesser tendon damage; however, there is not a single cause behind these injuries, but rather a complex multifactorial interaction of both extrinsic and intrinsic factors.

According to Cook and Purdam (2009), the load is the greatest pathologic component for the tendon, but how this load affects the tendon varies according to how it is modulated by diverse individual extrinsic and intrinsic factors. Therefore, it is clear that not all tendons will react in the same way to the same stimulus (Cook & Purdam, 2009).

2.3.4.1. Extrinsic risk factors

Loading/fatigue: considering that the tendon is a living tissue in which different cells live in a highly organized structure (Shepherd & Screen, 2013), there is a close relationship between structural changes and the molecular response exhibited by the tendon by way of mechano-transduction. It is to be expected, then, that the accumulated damage from excessive loads would produce structural changes as a result of modifications to the ECM, thereby compromising the function of the tendon (Andarawis-Puri & Flatlow, 2011).

Fatigue can be considered a progressive, localized structural damage to a material that is subjected to cyclical loading, dependent on the exposure time (Magnusson et al., 2010; Shepherd & Screen, 2013). In a review by Shepherd et al. in 2013, different protocols for producing fatigue in the tendon were evaluated, organized according to the model used (in vivo, ex vivo, or in vitro) (Shepherd & Screen, 2013). In this review, the authors highlight the diversity of protocols

and results according to the model used and the need to unify criteria in order to draw conclusions. Despite this lack of agreement in results, there are indications that at low levels of fatigue, small structural changes can be observed in the architecture of collagen fibrils, while changes in mechanical properties can be observed at higher levels of fatigue (Shepherd & Screen, 2013).

Lack of rest: it has been suggested that a lack of rest post-exercise (24 hours) can lead to an inadequate restoration of collagen levels post exertion, which can leave the tendon vulnerable to injury (Magnusson et al., 2010). However, no definitive conclusions can be drawn on the subject because of the lack of related scientific literature.

Immobilization: due to the properties of the tendon, absence of an adequate load, as in the case of immobilization, has a negative effect on its structure (Wang et al., 2012). It has been observed that depriving the tendon of an adequate load produced changes in its composition, showing structural modifications in both shape and number of cells, as well as the alignment of collagen fibers. Furthermore, the absence of loading increases catabolic mechanisms, causing a decrease in collagen synthesis (Andarawis-Puri et al., 2015; Kjaer, 2004), progressive degeneration (Wang et al., 2012) and ultimately atrophy (Thornton & Hart, 2011). Immobilization also reduces water and PG content in the tendon, increasing the number of reducible cross-links in collagen (Sharma & Maffulli, 2006) and agrecan (Killian et al., 2012).

In a study by Thornton et al. (2010), a protocol in which the Achilles and supraspinous tendons in rats were deprived of loading led to an increase in expression of MMP-13 and TIMP-2. This increase in MMP-13 is also characteristic of supraspinous tendon tears in humans. High levels of MMP-13 may therefore be related to the progression of tendinopathy (Thornton & Hart, 2011); however, due to the lack of human studies, a direct relationship cannot be determined.

Despite the observation of these modifications, due to the low metabolic rate of the tendon, these modifications present with longer periods of immobilization compared to other tissues (Kjaer et al., 2009; Sharma & Maffulli, 2006).

2.3.4.2. Intrinsic risk factors

Age: the risk of suffering tendon injuries increases with age (Dunkman et al., 2013). Changes in the structure and biomechanics of tendons have been observed with increasing age, including a reduction in the number of tenocytes (Yu et al., 2013), and modifications in the structure and alignment of fibers (Dunkman et al., 2013). Changes in the behavior of tenocytes can be noted, promoting an alteration in migration and rate of proliferation, which is associated with reduced efficiency in repair processes and an increase in tendon injuries (Frizziero et al., 2014). A decrease in the number of tendon stem cells is also observed, as well as reduced potential for renewal (Ruzzini et

al., 2014). Finally, aging leads to a decrease in tendon rigidity, making them less capable of transferring force (Dunkman et al., 2013).

Sex: generally speaking, women have a higher risk than men of suffering exercise-related musculoskeletal injuries (Miller et al., 2007). However, regarding incidence of tendon injuries, male sex is considered an associated risk factor (September et al., 2012). Nevertheless, a study published by Miller et al. reported that collagen synthesis is lower in women, both at rest and 72 hours after performing an exercise protocol, which promotes risk of injury (Miller et al., 2007). Considering the triggering mechanism of tendon injury, we can see that athletic overuse injuries have a higher incidence in men, while occupational overuse injuries are seen more in women, particularly over age 30 (Thornton & Hart, 2011).

Obesity: the World Health Organization recommends the standard body mass index (BMI) classification to establish scales of overweight and obesity; according to the scale, subjects with a BMI between 25 and 29.9 are overweight, while obesity corresponds to a BMI greater than 30. Other measurements used, such as waist/hip ratio, are related to a pathological distribution of body fat.

Recently, obesity has been proposed as a risk factor for tendon injuries (Franceschi et al., 2014). Being that obesity is a changeable factor, it is necessary to understand the mechanisms through which

obesity affects the tendon. Several reviews have been carried out on the subject in a fairly short period of time. In 2009, Gaida et al. observed that, generally speaking, subjects who presented tendon abnormalities had significantly higher levels of adiposity than the controls in each group. The justification found in this review was based in two fundamental changes: on the one hand, mechanical changes induced by an increase in weight over load-bearing tendons, thereby explaining that the pathology of the lower limbs could be influenced by this excessive load; on the other hand, the higher incidence in tendons which were not subjected to large loads suggests there is a metabolic mechanism implicated. This mechanism could be due to peptides secreted by adipose tissue which could influence the structure of the tendon (Gaida et al., 2009).

The review done in 2014 by Franceschi et al. likewise related obesity with increased risk of suffering tendinopathy by way of mechanical and metabolic pathways. It is also suggested that there is a lower ability of tendons to resist stress and repair damage in obese subjects (Franceschi et al., 2014). A new contribution from Franceschi et al. (2014) is the hypothesis that a prolonged state of low, systemic inflammation as in the case of obesity could enhance the failure in inflammatory response after an acute tendon injury, creating a predisposition to chronic injuries.

Menopause: due to the hormonal changes and systemic loss of estrogens, menopausal women present higher levels of degenerative changes in tendons which could affect their ability to support certain physiological loads (Thornton & Hart, 2011).

Contraceptives: regarding the use of oral contraceptives (OC) and predisposition to tendinopathy, there is no current consensus. Several studies have related the use of OC with increased risk of tendon injury fundamentally due to the fact that administration of oral estrogens reduces serum levels of IGF-1, while increasing concentrations of IGF binding proteins. This reduced bioavailability of IGF-1 translates to less collagen synthesis in the tendon, connective tissue in muscles and bones. Furthermore, administration of OC could be related to a reduced tendon response to mechanical load (Hansen et al., 2009).

However, despite a large portion of the literature pointing in that direction, the same authors in a 2013 study did not find significant differences in tendon morphology, strength of collagen bridges, biomechanical properties or collagen content when comparing athletes using OC and those who did not use OC (Hansen et al., 2009). The diversity of results could be due, in part, to the different methodology used in each study; for this reason, it is necessary to unify criteria to establish the real effects of OC on the tendon.

Plasma glucose levels: it has been proposed that elevated fasting plasma glucose levels (greater than 100 mg/dl) may be related to risk of suffering tendon pathology. Specifically, in a study by Longo et al. in 2009, it was shown that there was a relationship between elevated glucose levels (even within the normal range) and risk of suffering a rotator cuff tear (Longo et al., 2009). However, due to the scarcity of studies on the subject, any relationships between this factor and risk of tendinopathy should be approached cautiously.

Diabetes: among risk factors related to tendinopathy, diabetes is one of the most-studied and well-understood (Abate et al., 2013). According to Abate et al. in a review from 2013, the changes caused by diabetes lead to an increase in the density of collagen fibrils, accompanies by a reduction in the number of fibroblasts and tenocytes per unit area. A reduction in elastic fibers was also observed, as well as reduced blood flow due to the presence of fewer capillaries, the latter of which is most characteristic in elderly subjects (Abate et al., 2013).

ABO blood group: several studies have analyzed the correlation between blood group and predisposition to tendon injury; however, the literature is not conclusive. In the Achilles tendon, some studies show that individuals with Type O are more likely to suffer tendon tears as well as paratendonitis (Jozsa et al., 1989). However, more recent studies do not support this association (Maffulli et al.,

2015). A recent study by Lee et al. in 2015 found that subjects who had suffered a rotator cuff tear were more likely to have Type O blood.

Laxity: certain alterations in musculoskeletal flexibility, both an increase and decrease, have been associated with an increased risk of suffering musculoskeletal injuries (Collins et al., 2009). However, there is not much consensus regarding how this property really affects predisposition. As will be discussed in section 1.5.6 "Genetic Polymorphisms", a relationship was found between decreased range of movement in subjects with genotype TT for COL5A1 and an increase in risk of suffering tendinopathy of the Achilles tendon (Brown et al., 2011). On the other hand, the articular hypermobility associated with Ehlers-Danlos syndrome is associated with structural changes of the tendon and decrease in its rigidity (Nielsen et al., 2014).

Fluoroquinolones: fluoroquinolones are a family of antibiotics used in the treatment of different respiratory, cutaneous and sexually transmitted infections (Lewis & Cook, 2014). Examples include norfloxacin, ciprofloxacin, ofloxacin, levofloxacin, etc. In a review by Lewis and Cook in 2014, the influence of these medications was analyzed with relation to development of tendinopathies. It was noted that, although the exact mechanisms of action of these drugs on the tendon are not entirely clear, we can infer that they produce alterations in the tendon through three pathways: ischemia, degradation of the tendon matrix, and alteration of tenocyte activity. All of these

pathways are related to an increase in MMPs; specifically, several studies have observed an increase in MMP3 in the Achilles tendon with the consumption of ciprofloxacin. The most reported symptom of tendinopathy caused by this medication is pain, generally sudden onset, while localization is in the Achilles tendon in 95% of cases (Lewis & Cook, 2014).

Tobacco: tobacco has been associated with increased number of musculoskeletal injuries, such as bone fractures, osteoarthritis or back pain. Aside from these injuries, in recent years a positive relationship has also been found between cigarette consumption and risk of tendon pathology (Lee et al., 2013; Abate et al., 2013). Specifically, tobacco consumption has been associated with presence of persistent shoulder pain and tendinopathy, primarily on the dominant side; a positive correlation between distal biceps tendon tears and tobacco consumption has also been found in humans, and nicotine has been shown to negatively impact Achilles and rotator cuff tendon healing in animal models (Abate et al., 2013).

2.3.4.3. Genetic polymorphisms and tendon pathology risk

Recently, genetic factors have been attributed with important effects on tendon pathology. Certain genetic variations are considered to increase the risk of suffering greater damage and consequently increasing the chances of developing tendinopathy (Juneja & Veillette,

2013; Kim et al., 2014; Magra & Maffulli, 2008; Magra & Maffulli, 2007).

Currently, two pathways are known by which these genetic modifications affect the structure of the tendon. On the one hand, genes coding for specific structures of the ECM, and on the other hand, genes that are related to the metabolism of the tendon. Regarding genes associated with the structure of the ECM, genes related to regulation of collagen and tenascin-C are primarily studied, given that their modulation is necessary to conserve the properties of the tendon (Mokone et al., 2006; September et al., 2012; Magra & Maffulli, 2008).

Keeping in mind the main properties of collagen, we can observe that these properties are conserved throughout the species, such that each polypeptide α chain is coded by a specific gene. Therefore, a mutation in these genes could produce musculoskeletal changes (among others), of varying degrees of seriousness depending on the genetic mutation.

Certain mutations in collagen genes have been associated with alteration or loss of function. Mutations in COL5A1 and COL11A1, for example, are seen in certain types of Ehlers-Danlos syndrome (Altinisik et al., 2015) and Marshall syndrome (Griffith et al, 1998) respectively. Mutations in COL11A2 are associated with

otospondylomegaepiphyseal dysplasia (Melkoniemi et al., 2000) and Weissenbacher-Zweymüller syndrome (Pihlajamaa et al., 1998). Both COL11A1 and A2 have been associated with Stickler syndrome (Acke et al., 2014). All of these disorders can impact joint extensibility or cause collagen-related skeletal abnormalities.

While the most serious pathologies associated with Mendelian disorders, such as osteogenesis imperfecta, Ehlers-Danlos syndrome and achondroplasia have been studied in more depth, there are not many studies done on the mild effects of these genes and their consequences on collagen regulation. There is an emerging current that points to small disorders in the expression of these genes explaining predisposition to certain pathologies, including tendinopathy. Three genes of particular interest are COL5A1, COL11A1 and COL11A2.

The gene COL5A1 codes for the α1 chain of collagen V and is found on chromosome 9 in humans. Collagen V is responsible for giving structure and lateral growth to tendon fibrils; as such, an alteration in this protein may affect the structure of the tendon (September et al., 2012). It also has an important function in regulation of fibrillogenesis, as well as in the tendon's ability to support tension (Magra & Maffulli, 2007).

The variant of this gene, single nucleotide polymorphism (SNP) rs12722 produces two different alleles, C and T, thus shaping

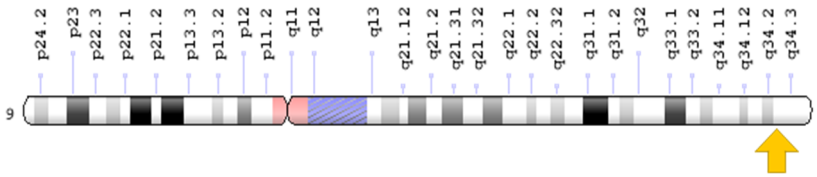

Figure 15. Location of COL5A1 gene in humans. Source: U.S. National Library of Medicine Genetics Home Reference.

different genotypes (September et al., 2012, Connizzo et al., 2015). While genotype CC has been associated with lower risk of chronic Achilles tendinopathy (Mokone et al., 2006), genotype CT is associated with less elasticity in the lower extremity (Collins et al., 2009) and genotype TT is associated with lower range of mobility, particularly in elderly subjects (Brown et al., 2011), and greater risk of chronic Achilles tendinopathy (September et al., 2012) due, in part, to reduced elasticity of the tendons. While in these genotypes both copies of COL5A1 are functional, there is the rare possibility that mutations are produced within one of these copies, potentially causing Ehlers-Danlos syndrome (September et al., 2012).

O'Conell et al. proposed in a 2013 study the progression of disorders associated with COL5A1 mutations according to the seriousness of the affectation. They observed that, while mutations of one or both copies of COL5A1 cause serious alterations characteristic of Mendelian disorders, different genotypes of COL5A1 lead to changes in phenotype, subject to exposure to certain factors that may

enhance risk of developing certain pathologies (O'Conell et al., 2013). A 2015 study by Altinisk et al. found a significant correlation between COL5A1 rs12722 and rs13946 variants and risk of lateral epicondyle tendinopathy (tennis elbow). The authors reported a protective effect of the CC genotype in development of elbow tendinopathy for the rs12722 SNP, and for TT genotype in SNP rs13946 (Altinisk et al., 2015).

An overview of the posttranscriptional relationships between the COL5A1 gene and other components of the ECM is as follows: COL5A1 gene expression may be increased by interleukin-6 (IL-6), which is induced through increased mechanical loading of the tendon and by interleukin-1β (IL-1β), which is also induced through loading; an increase in COL5A1 expression may lead to increased risk of tendinopathy. Type V collagen as part of the tendon ECM may also be degraded by matrix metalloproteinases (MMPs) which are activated by IL-1β, thus increasing the risk of tendinopathy. IL-6 can also trigger the synthesis of an apoptotic mediator which leads to apoptosis of tenocytes and, again, increased risk of tendinopathy (Brown et al., 2017).

Genes affecting collagen XI have also been studied because of their homology and joint participation in the processes of fibrillogenesis. COL11A1 is located on chromosome 1 and codes for the α1 chain of collagen XI, while COL11A2 is located on

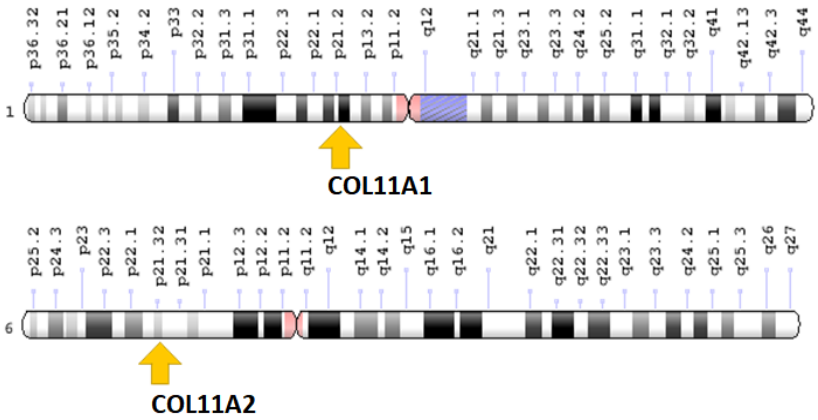

Figure 16. Location of COL11A1 and COL11A2 genes in humans. Source: U.S. National Library of Medicine Genetics Home Reference.

chromosome 6 and codes for the α2 chain. A 2013 study by Hay et al. analyzed COL11A1 rs3753841 (T/C), COL11A1 rs1676486 (C/T) and COL11A2 rs1799907 (T/A) to establish their independent association with chronic Achilles tendinopathy without being able to establish a relationship, which suggests that they interact with other genes related to collagen V (Hay et al., 2013).

More recently, researchers have become interested in epigenetic risk factors that might predispose an individual to tendinopathy. Epigenetic factors are elements which influence gene expression without involving changes in DNA sequence. To date, the epigenetic control factor that has received the most attention in this area of research is DNA methylation in certain genes that have been shown to have differential levels of expression in tendinopathy patients

compared to controls. It is known that an individual's methylation profile may change in response to physical activity. However, somewhat surprisingly, the role that DNA methylation plays as an epigenetic factor in relation to human tendinopathy has only recently begun to be investigated (El Khoury et al., 2018).

DNA methylation occurs when a methyl group binds to a cytosine nucleotide base, converting it to 5-methylcytosine. This takes place primarily in CG-rich regions, which are typically located in the promoter regions of genes. DNA methylation tends to down-regulate gene expression by interfering with the ability of transcription factors to access their respective binding sites on these genes. In this way, DNA methylation can profoundly affect transcription levels such that an epigenetic mode of inheritance might significantly modify the effect of genotype upon phenotype. Methylation may result from either directionally programmed changes, as part of the aging process, or from spontaneous alterations attributed to environmental factors.

A recent study by El Khoury et al. (2018) examined the influence of DNA methylation on two genes related to components of the tendon ECM: TIMP2 and ADAMTS4. The ADAMTS4 gene encodes a protein that degrades aggrecan and a number of other components of the ECM. Its expression has been found to increase in knee osteoarthritis, which might exacerbate the condition by

contributing to the elevated degradation of proteoglycans. ADAMTS4 mRNA expression levels have been shown to differ in ruptured Achilles tendons compared to normal and tendinopathic tendons. The expression of ADAMTS4 likely plays an important role in the tendinopathic process, but our understanding of how its expression is controlled in tendinopathy is still unclear. We do know, however, that it is under epigenetic control, and loss of methylation has been shown to be responsible for the upregulation of ADAMTS4 protein in osteoarthritic cartilage.

El Khoury et al. (2018) examined the methylation profiles at four and six sites on the TIMP2 and ADAMTS4 genes, respectively, in participants with patellar tendinopathy, and compared these profiles with a control group. Their results suggest that methylation is not involved in TIMP2 expression in the process of patellar tendinopathy. This was unexpected given that a previous study had shown differential expression of TIMP2 in patients with Achilles tendinopathy (Karousou et al,, 2008). It is possible that methylation differences between the tendinopathy and control groups is occurring at a CpG site on that gene that was not one of the four sites analyzed in their study. However, they did find a significantly higher level of methylation of one CpG site on the ADAMTS4 gene. Because of the location of this site relative to an important binding site for transcription factors known to regulate ADAMTS4, they propose that methylation at this site could interfere

with the binding of these transcription factors and thus alter the expression of the gene. Reduced expression of ADAMTS4 has previously been associated with an increase in the proteoglycan aggrecan, and patellar tendinopathy patients in El Khoury et al.'s study were found to have an increased accumulation of proteoglycans, including aggrecan. However, there was no significant difference in ADAMTS4 levels between the pathologic and control groups.

3. Summary of justification, aims and hypotheses

Based on the lack of scientific literature on the subject, the mechanisms leading to tendinopathy of the elbow are not clearly understood. Due to the multifactorial nature of the pathology, it is complicated to point out a single factor as the principal cause. However, in sports medicine, the risk of injury is highly variable in the face of very similar stimuli, suggesting the importance of individual intrinsic factors such as genetic variations.

We have proposed the present study given the need to be able to stratify athletes according to the risk of tendinopathy and in so doing be able to carry out individualized preventive and rehabilitative programs, thereby minimizing the impact of an injur y with a relatively high rate of incidence.

We hypothesize that there is a positive association between SNPs COL5A1 rs12722, COL11A1 rs3753841, COL11A1 rs1676486,

and COL11A2 rs1799907 and the risk of suffering tendinopathy of the elbow, as well as an association between other biological markers and this pathology. Understanding the multifactorial nature of tendinopathy, we hypothesize that genetic factors will have a significant influence on elbow tendinopathy but may not be the most influential of all the biological factors analyzed.

The aims of the present study are to examine the association between known and suspected intrinsic risk factors and elbow tendinopathy:

1. Analyze the influence of SNPs COL5A1 rs12722, COL11A1 rs3753841, COL11A1 rs1676486, and COL11A2 rs1799907 on the risk of suffering elbow tendinopathy.
2. Analyze the influence of other demographic and anthropomorphic variables such as sex, body composition, BMI, glucose levels, and joint laxity on elbow tendinopathy.
3. Analyze the influence of certain lifestyle factors on risk of elbow tendinopathy.
4. Assess the relative importance of each of these factors as they relate to risk of suffering elbow tendinopathy.

II. MATERIAL AND METHOD

1. About the study

1.1. Study design and location

The present study is a case-control study which aims to evaluate the association between specific genetic polymorphisms and risk of tendinopathy of the elbow, as well as other biomarkers which may be risk factors for this pathology.

Sample and data collection was done in the Clinical Research Laboratory of the University of Valencia Faculty of Physiotherapy, located at Calle Gascó Oliag 5, 46010, Valencia, Spain.

1.2. Study participants

This study included 139 participants between the ages of 18 and 50 from the general population who participate in sports which involve loading of the elbow joint, such as swimming, volleyball, tennis, handball, water polo, baseball, softball, etc. Of the 139 selected participants, two had to be discarded because of degraded blood samples that prevented us from performing the genetic analysis. The final sample was composed of 137 participants.

The first contact with participants was through informational talks in the Faculties of Physiotherapy and Physical Activity and Sports Sciences, as well as meetings with different sports teams participating

in those sports indicated previously, where the nature of the study was explained, along with characteristics of participation, risks, benefits and implications. Those interested in participating were evaluated according to their possibility of inclusion as determined by the inclusion and exclusion criteria.

1.2.1. Inclusion criteria

The following were inclusion criteria for participation in the present study:

- Age between 18 and 50 years, limits establishing legal adulthood and that the tendons would not be very degenerated due to aging.
- Current participation in sports which involve stress to the elbow joint: swimming, volleyball, tennis, handball, water polo, baseball, softball, etc.
- Weekly participation in this sport of 4 hours or more.
- Continuous participation in this sport for 2 years or more.
- Not meeting any exclusion criteria.

1.2.2. Exclusion criteria

Exclusion criteria for both groups in the study were:

- Previous surgery on the elbow joint.
- Osteoarthritis of the elbow joint.

- History of subluxation or dislocation of the elbow, fracture of the humerus, radius or ulna.
- Taking medication in the past 6 months which affects the characteristics of the tendon (fluoroquinolones, corticosteroids).
- Pregnant women, because they experience hormonal changes and an increase in tissue laxity which may affect the results of this study.
- Inflammation in the joint diagnosed as rheumatoid arthritis or ankylosing spondylitis.
- Cancer.

1.2.3. Ethics and informed consent

All participants were informed in detail of the subject, nature, practical details and possible risks of the present study, as well as their ability to decline participation at any time.

The present study is based on the principles of the Declaration of Helsinki, good clinical practices guide and Spanish law as ethical guidelines for its realization.

We have also received the appropriate permissions from the Human Research Ethics Committee of the University of Valencia (H1409657453224), where it was concluded that the study respects the

fundamental principles established in the Declaration of Helsinki, the Council of Europe Convention regarding human rights, and the requirements established in Spanish law regarding biomedical research, protection of personal data, and bioethics.

After being selected, participants were seen two at a time in the laboratory, where they were informed of the experimental procedure and given the informed consent document to be signed.

2. Methods

2.1. Group allocation

Participants were distributed into two groups according to the presence or absence of pathology in any of the tendons of the elbow. To do this, a comprehensive ultrasound examination was performed by a physiotherapist technician specialized in ultrasound to evaluate the integrity of the tendons of the elbow, as well as evaluation of functionality and joint pain. All of this was accompanied by a history of each participant's lifestyle habits, clinical history, sports participation and family history.

Participants were sorted into the control group or pathological group based on the results of the ultrasound. Those who presented abnormality in any tendon in one or both elbows were assigned to the

pathologic group, while those who presented normal tendons were assigned to the control group.

Tendons were classified as normal if they did not present any type of change, and pathological if they presented an increase in size, hypoechoicity and/or evidence of partial or total tears.

2.2. Experimental protocol

The experimental protocol consisted of a series of tests to study the variables of interest, all performed in a single session by a team of three qualified professionals in their fields:

- Certified ultrasound technician and Doctor of Physiotherapy with substantial experience in clinical ultrasound tests.
- Nurse and licensed physiotherapist with experience in blood draws.
- Physiotherapist certified in Physical Activity and Sports Sciences, with level 1 ISAK certification in Anthropometry and experience in the field.

The variables of the study can be categorized as:

- Demographic information: age, sex, dominant hand, ethnic group, and anthropometric data including weight, height, BMI, and waist circumference.

- Sports participation: relevant information regarding the different athletic activities done in recent years, weekly participation in the last three months, six months, year and before that year.
- Personal and family history: presence of systemic illness, previous injuries, medications, tobacco use.
- Hypermobility and laxity: measured as degree of articular mobility in different joints using the Beighton test.
- Clinical examination of the elbow: evaluation of pain, function and mobility.
- Ultrasound examination: evaluation of the integrity of the tendons of the elbow.
- Genetic analysis: analysis of genetic markers that have been previously associated with different tendon pathologies and published in the recent literature: COL5A1 rs12722, COL11A1 rs1676486, COL11A1 rs3753841, and COL11A2 rs1799907.
- Fasting glucose analysis.

As stated previously, all data corresponding to these variables were collected in a single laboratory session. Different questionnaires and tests were used appropriate for each variable, which will be explained in detail in subsequent sections.

Once the data from the experimental protocol were collected, they were recorded and analyzed to classify each subject according to the presence or absence of pathology and grouped accordingly into the

pathologic or control groups. Finally, statistical analysis was performed to obtain the relevant results and evaluate their significance.

2.2.1. Anthropometric data collection

A template for recording anthropometric data was used to record the different measurements performed, which included the following:

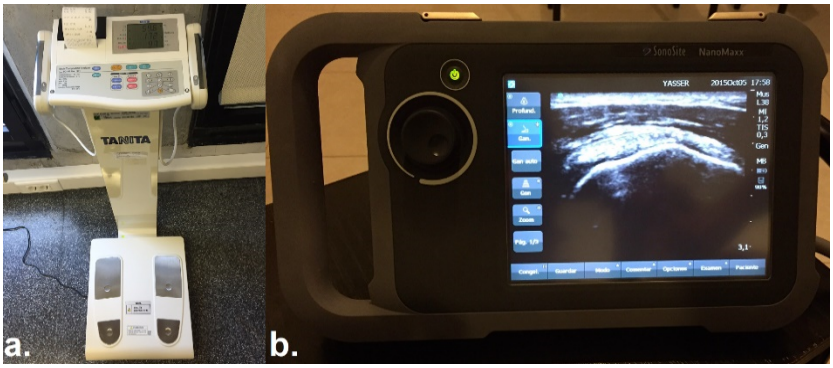

Figure 17. (a) Body composition analyzer: TANITA model BC 418 MA, (b) Portable touchscreen ultrasound system: NanoMax by FUJIFILM SonoSite with Color Power Doppler technology for high quality diagnostic imaging.

– Body composition: measured using a TANITA bioelectric impedance analysis (Figure 17a), where weight and percent body fat were measured. Subjects were fasting for 10 hours, without socks or shoes. Subjects stood in bipedestation with arms relaxed and holding the handles, keeping the palms in contact with the sensors, while the feet were in contact with the four corresponding electrodes (two on the heel and two on the metatarsal).

- Height: measurement at maximum extension with a measuring board. The subject was in bipedestation with heels together and inhaled deeply, maintaining their head on Frankfort's plane.
- Body mass index (BMI): calculated using measurements from the TANITA analysis and height measurement, using the following formula:

$$\text{BMI} = \text{weight in kg}/(\text{height in m})^2$$

- Waist circumference: measured at the midpoint between the last costal arc and the iliac crest, at the narrowest part of the abdomen.

A test for range of mobility was also performed using specific tests to evaluate the maximum angle of mobility measured by goniometer, assessing possible restrictions in movement and differences between the dominant and non-dominant sides. Angles of elbow extension, flexion, pronation and supination were measured and compared to standard references.

For flexion-extension, the participant lay in dorsal decubitus position with the arm supported on a pillow alongside the body with the mobile arm of the goniometer aligned with the medial longitudinal line of the fifth metacarpal, at 0°, and the fixed arm aligned with the medial longitudinal line of the ulna (Figure 18a).

The participant then flexed the forearm up towards the bicep, and this movement was followed with the mobile arm of the goniometer to the point of full flexion, and this angle was recorded

(Figure 18b). Finally, the participant brought the forearm back down to the point of full extension (Figure 18c).

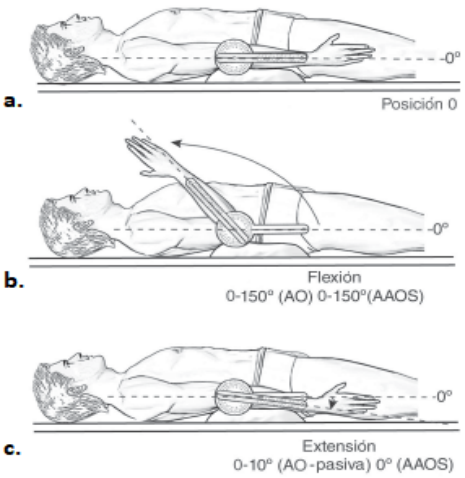

Figure 18. Goniometric measurement protocol for flexion-extension of the elbow joint, a) starting or 0 position, b) full flexion, c) full extension. Taken from Taboadela, 2007.

For pronation-supination, the participant was seated with the shoulder in 0 position and the elbow flexed at 90° to prevent rotation of the shoulder, with the forearm and wrist in 0 position (Figure 19a). The fixed arm of the goniometer was aligned parallel to the medial longitudinal line of the humerus, on the outside for pronation and on the inside for supination, with the mobile arm at 0°. To measure pronation, the participant turned the hand and forearm towards the midline of the body, and this movement was followed by the mobile arm of the goniometer, until the point of full pronation. The angle was recorded using the ulnar styloid process as a reference point (Figure

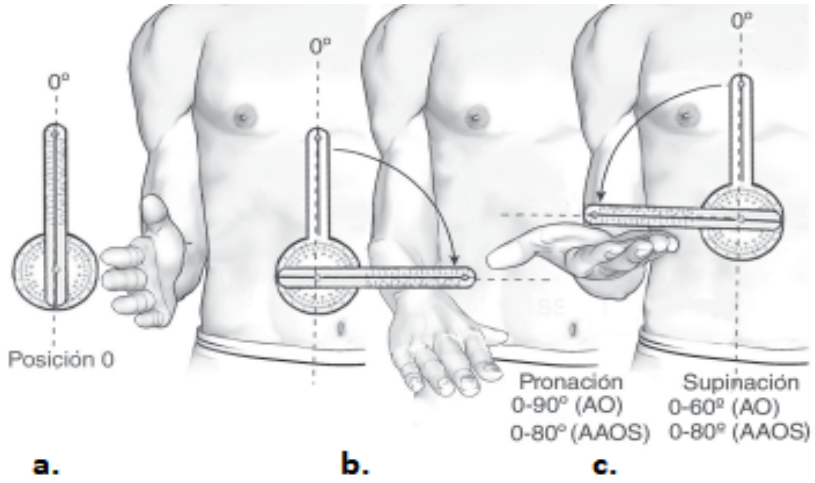

Figure 19. Goniometric measurement protocol for pronation-supination of the elbow joint, a) starting or 0 position, b) full pronation, c) full supination. Taken from Taboadela, 2007.

19b). To measure supination, the participant turned the hand and forearm away from the midline of the body, followed by the mobile arm of the goniometer, until the point of full supination. The angle was recorded using the radial styloid process as a reference point (Figure 19c.)

The Beighton test was used as a simple method to quantify articular laxity and hypermobility. It uses a system of 9 points according to the possibility of overreaching the normal limits of the joint being tested. The threshold which determines articular laxity is 4 based on the test's own recommendations; however, scores greater than 6 would have greater validity and correlation if compared with other tests for laxity (Collins et al., 2009).

2.2.2. Ultrasound examination of tendons

To evaluate the integrity of the tendons of the elbow, ultrasound (US) images of both elbows were taken by a physiotherapist with experience in ultrasound. Tendons were classified as normal or abnormal based on the presence of some abnormality. To perform the examination, US images were taken using the FUJIFILM SonoSite NanoMax US system (Figure 17b) of the lateral and medial sides of the elbow. A water-based gel was used to enhance visibility.

For examination of the common extensor tendon of the lateral elbow, the elbow is placed in flexion with the arm internally rotated. The transducer is then placed in the longitudinal axis (coronal plane), and the tendon can be visualized by placing the cranial edge of the transducer on the lateral epicondyle of the humerus, slightly oblique to the long axis (Figure a).

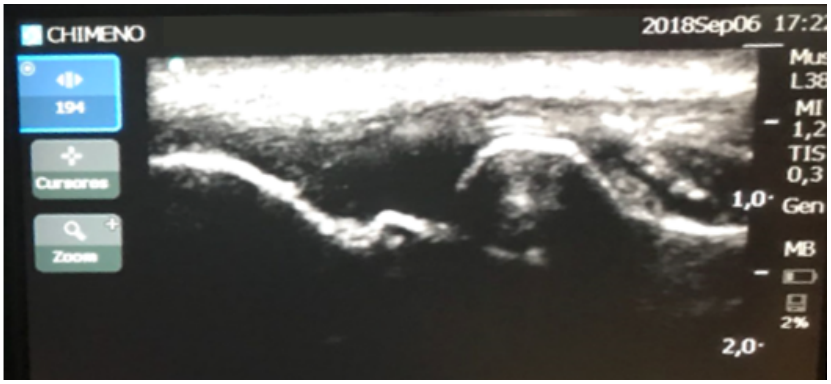

Figure 20 Ultrasound examination of the tendon of the lateral elbow. Own image.

To examine the common flexor tendon of the medial elbow, the elbow is placed in forceful external rotation and extension or slight flexion. The transducer is then placed in the long axis (coronal plane) with the cranial aspect placed over the medial epicondyle (Figure b).

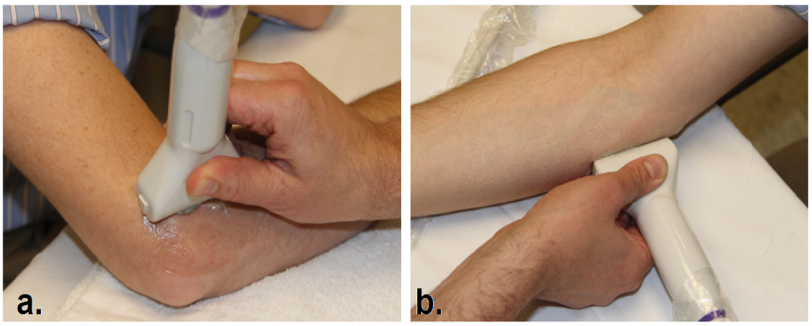

Figure 21. US transducer technique to visualize the tendons of the (a) lateral and (b) medial elbow. Taken from Konin et al., 2013.

2.2.3. Questionnaires

Several different questionnaires were used to collect relevant clinical history and evaluate functionality and pain.

– General questionnaire for tendon injuries: age, sex, lifestyle habits, sports participation, medical history, family history, previous tendon injuries, medications, ABO blood group.

- The Mayo Elbow Performance Index questionnaire was used to score pain, range of movement, stability and functionality of the elbow, with final possible scores ranging from 5 to 100 points and higher scores corresponding to better function; scores between 90 and 100 are considered "excellent functionality" (Longo & Franceschi, 2008).

- The Level of Shoulder Activity questionnaire was also used since, despite its name, it provides an assessment of the entire upper limb and not just the shoulder. It consists of a main section which collects information about the frequency with which the upper limb is used in different situations, with higher scores corresponding to greater activity levels. The remainder of the questionnaire is to determine whether the respondent participates in a contact sport or throwing sport and the level of competitiveness of the respondent's sports participation (Brophy & Beauvais, 2005).

- DASH (Disabilities of the Arm, Shoulder and Hand): a specific questionnaire which evaluates quality of life related to problems of the upper limb. It consists of three sections in which the ability to perform activities of daily life are scored from 1 to 5, with increasing values for greater intensity of symptoms. The first obligatory section contains 30 questions, and the optional second and third sections of occupation and high performance

athletes/musicians contains 4 questions. Scores of each item are totaled to obtain a total score between 30 and 150 points, which is converted to a scale of 0 (best possible score) to 100 (worst possible score). The optional sections are scored separately following the same method.

2.2.4. Blood draw and handling

To determine the polymorphisms related to tendinopathy, a blood sample of approximately 4.5 ml was taken via venipuncture from the forearm vein and extracted in Vacutainer EDTA tubes. Blood samples were stored at 4°C during the experimental protocol until subsequent transport and storage. Subjects were in fasting for 8 hours at the time of extraction for correct measurement of fasting glucose levels.

After finishing the protocol, the samples were transported in a cooler at 4°C to the Central Unit of Medical Research (UCIM) of the Faculty of Medicine at the University of Valencia, where they were stored in a Controltecnica Instruments® ultrafreezer at -80°C. An internal communication network was established among all users of the freezer, guaranteeing a secure emergency protocol in case the samples had to be moved to another freezer for technical reasons.

Once the data collection phase was finished, all samples were moved under optimal conditions to the Genetics and Molecular

Biology Unit at La Ribera University Hospital in Alzira (Valencia), where the genetic analysis was performed.

2.2.5. Genetic analysis

DNA extraction was performed using the standardized procedures. Real-time PCR (polymerase chain reaction) technology was used to perform this analysis, which combines amplification and detection in a single step by correlating the PCR product of each cycle with fluorescent signal intensity.

To determine the genotype of the selected polymorphisms in the blood samples, a TaqMan SNP genotyping analysis (Applied Biosystems; Foster City, California, United States) was performed using the Real Fast 7900HT real-time PCR system (Applied Biosystems). Genotype results were reproduced for each subject in triplicate in independent tests.

2.3. Statistical analysis

2.3.1. Power study and sample size

To calculate sample size, the software G*Power 3.1 was used, establishing the one-factor ANOVA as the type of test to use, performed a priori with an alpha of 0.05 and beta of 0.80.

Based on the results of this analysis, the number of subjects necessary to obtain a sample representative of the population of interest was 128, which is in line with the sample used in a recent study published by Salles et al. (Salles et al., 2015) in which the association of tendinopathy in different joints was analyzed with the presence of certain genetic polymorphisms in a population of 138 professional volleyball players.

2.3.2. Data analysis

Mathematical software Matlab®, the open-source program R, and SPSS® were used as tools for data analysis in this study.

Different statistical tests were employed according to the number and types of variables. To analyze the relationship between the different genotypes and laxity, a one-factor ANOVA was used as a parametric method, as well as a Kruskal-Willis as a non-parametric method. As there were significant differences, other tests such as the Tukey test were later applied to determine where the differences were found.

For the remaining analyses, the chi-squared test was used to relate discrete and group variables, and Wilcoxon's test to establish relationships between the continuous and group variables. Additionally, for variables that implied a significant difference, the

Fisher post hoc (LSD) was used. Correlation coefficients, Cramer's distance, and V distance depending on the type of variable.

2.3.2.1. Machine learning

Random forest test: For several decades now, improvements in computational systems have increased the number of machine learning algorithms available to us and has led to the development of new machine learning techniques that allow us to obtain different classification models. One of the first to be used was the decision tree, a way to represent a set of rules with a hierarchical and consecutive structure that recursively divide the data (Murthy, 1998). Each rule is found in a decision node that indicates which decision to take as a function of the value of a variable. From this type of classification system comes the random forests, which consist of a combination of decision trees in which the combination of each one offers the most popular class according to the data. According to Lantz (2013), these models select only the most important variables and can be used in massive datasets (many variables or records).

In our work, we used this machine learning technique to determine which of our many variables (demographic, anthropometric, genetic, lifestyle, etc.) were the most important in predicting the presence of elbow tendon pathology.

Self-organizing maps: Another machine learning method that is used is the neuronal network, which involves the evolution of the perceptron—an artificial neuron—and its integration in a network system; in other words, the interconnection of the different neurons (Lantz, 2013). In this way, we have the possibility to create a linear or nonlinear model, which is an advantage over inherently nonlinear systems. From these neuronal networks emerged a special classifier that is used as a tool to visualize data: self-organizing maps or SOMs, where the neurons are found in the node of a mesh (map) that, normally, is one- or two-dimensional. The objective of these maps is to transform a given one-dimensional entry signal pattern into one or two dimensions within a discretized map and to illustrate this transformation in a way that is topologically organized (Haykin, 2009).

In our study, these SOMs have been very useful to jointly visualize the respective associations among a large number of different variables, including each variable's relationship (or lack thereof) with elbow tendon pathology. Given the characteristics of the sample and number of subjects, a map was applied which includes those variables related to the anthropometric and metabolic data in addition to the results obtained from the DASH questionnaire.

III. RESULTS

Based on US examination of the study population, 73.72% of participants showed normal tendon condition in all tendons of the elbow joint (no tendinopathy), while the remaining 26.28% of participants showed pathologic changes in the elbow tendon (tendinopathy). As a result, 36 participants were assigned to the pathological or case group, and 101 were assigned to the normal or control group.

Statistically significant associations were found for several of the variables studied, including genetic factors, quantitative biological factors and non-genetic categorical factors, as well as some tendencies that were not statistically significant but suggest that a relationship may exist. Machine learning tests revealed additional information about relationships between elbow tendon pathology and the variables studied, as well as relationships between the different variables themselves.

1. Characteristics of the study population

1.1 Anthropometric data

The study population was made up of 77 men and 60 women, with a mean age of 23 ± 5.5 years. Anthropometric data for the case and control groups are shown in Table 1.

Table 1. Anthropometric data of elbow tendon pathology cases and controls

	Cases (n=36)	Controls (n=101)
Sex, n (%)		
Male	18 (50.0)	59 (58.4)
Female	18 (50.0)	42 (41.6)
Age, mean ± SD (range)	23.1 ± 4.7 (18-35) years	23.1 ± 5.8 (18-44) years
BMI, mean ± SD (range)	24.7 ± 3.6 (20.5-36.5)	21.9 ± 2.8 (17-30.9)
Height, mean ± SD (range)	175.9 ± 10.8 (157-199) cm	174.0 ± 8.0 (152-190) cm
Weight, mean ± SD (range)	76.9 ± 15.8 (51.7-110.6) kg	66.8 ± 11.4 (44.4-96.2) kg
Waist circum. mean ± SD (range)	79.0 ± 9.6 (61.5-109.0) cm	73.8 ± 8.3 (58-96) cm
Dominant hand, n (%)		
Right	32 (88.9)	92 (91.1)
Left	2 (5.6)	6 (5.9)
Ambidextrous	2 (5.6)	3 (3.0)
Side of tendinopathy, n (%)		
Dominant only	20 (55.6)	N/A
Non-dominant only	5 (13.9)	N/A
Bilateral	11 (30.6)	N/A

SD = standard deviation, BMI = body mass index (weight in kg/height in m^2)

1.2 Ultrasound results

Thirty-six participants showed tendon abnormalities in at least one tendon of the elbow upon ultrasound imaging. The distribution of these abnormalities across the four tendons (left and right common extensor tendons, attaching at the epicondyle, plus left and right common flexor tendons, attaching at the trochlea) is shown in Table 2.

Table 2. Abnormal tendons on US imaging in case group

Abnormal tendon(s) on US imaging	n (total = 36 cases)
Right epicondyle only	7
Left epicondyle only	2
Right epitrochlear only	8
Left epitrochlear only	4
R. Epicond. + L. Epicond.	1
R. Epitroch. + L. Epitroch.	4
R. Epicond. + R. Epitroch.	4
L. Epicond. + L. Epitroch.	0
R. Epicond. + L. Epitroch.	1
L. Epicond. + R. Epitroch.	2
R. Epicond. + L. Epicond. + L. Epitroch.	1
R. Epicond. + R. Epitroch. + L. Epitroch.	1
L. Epicond. + R. Epitroch. + L. Epitroch.	1

R. Epicond. = right epicondyle; L. Epicond. = left epicondyle; R. Epitroch. = right epitrochlea; L. Epitroch. = left epitrochlea.

Thus, because the common extensor tendon attaches at the epicondyle and the common flexor tendon at the trochlea, we can say that 10 participants could be diagnosed as having tendon pathology characteristic of tennis elbow based on ultrasound imaging (7 on the right elbow, 2 on the left, and 1 bilateral pathology). Furthermore, 16 were diagnosed as having tendon pathology typical of golfer's elbow (8 on the right elbow, 4 on the left and 4 bilateral pathologies), and the remaining 10 participants had some combination of tendon abnormalities at both the epicondyle and trochlea tendons.

2. Questionnaires and functional tests

The tendon pathology group as determined by abnormal tendon structure on ultrasound imaging had DASH Sports and Occupational scores that were significantly higher than those of the normal group (p=0.03 and p=0.005, respectively), reflective of higher perceived pain and lower perceived functionality in the case group.

We believe that this supports our use of structural abnormalities upon ultrasound imaging as our diagnostic criteria for group assignment, especially knowing that symptoms are not reliably associated with tendon pathology.

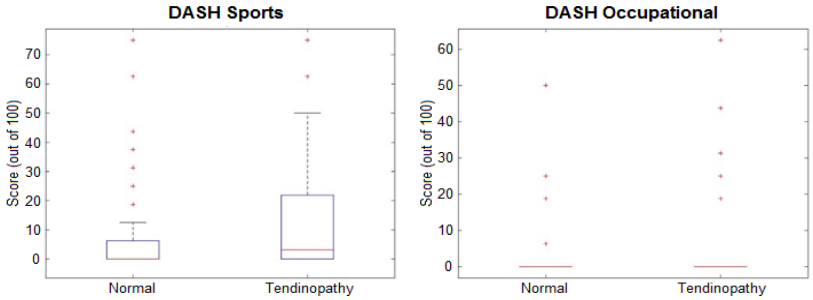

Figure 21. Significant differences in DASH scores (sections 2 and 3) between the normal and tendon pathology groups, with the tendon pathology group having higher scores (greater perceived pain and disability).

For categorical variables, based on chi-squared analysis, two non-genetic factors were found to have a statistically significant relationship with elbow tendinopathy: participation in throwing sports (p=0.03) and previous incidence of tendinopathy (p=0.02) (Figure 22). In throwing sports, subjects participating at an amateur competitive level were most likely to have tendinopathy, compared to recreational

and elite/professional levels. With previous incidence of tendinopathy, a history of tendinopathy in either the dominant side or bilaterally were associated with increased risk of suffering current elbow tendinopathy

As for contact sports, no significant relationship was found with the presence of elbow tendinopathy. However, there was a statistically significant association between participation in contact sports and instability in both the right and left elbow joint (p=0.030 and 0.018, respectively). There was also a significant relationship between sex and instability of both elbows, with women being more likely to present elbow instability (p=0.006 for right and p=0.008 for left elbow).

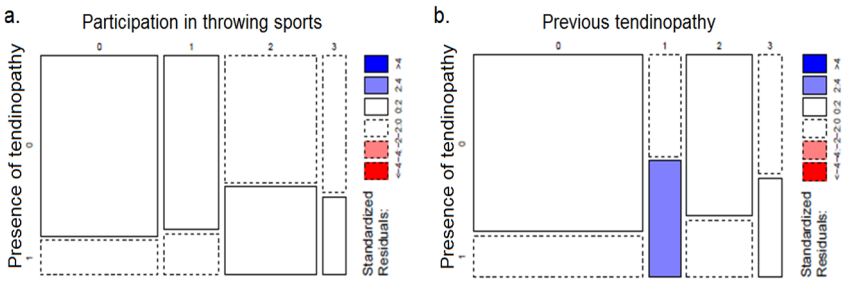

Figure 22. Mosaic of (a) participation in throwing sports and elbow tendinopathy, and (b) previous tendinopathy and elbow tendinopathy. Presence of tendinopathy: 0 = absence, 1 = presence. Throwing sports: 0 = none, 1 = recreational, 2 = amateur competitive, 3 = elite/professional. Previous tendinopathy: 0 = none, 1 = dominant side, 2 = non-dominant side, 3 = bilateral.

A Mann-Whitney U test did not find a statistically significant relationship between joint laxity and elbow tendon pathology (p=0.77). However, a one-way ANOVA did reveal significant differences in laxity among the three genotypes for one of the SNPS studied,

COL5A1 rs12722 (p=0.027). The CT genotype had a higher mean laxity than the CC and TT genotypes (3.23 vs. 1.25 and 2.70, respectively).

Upon discovering the significant relationship between SNP COL11A1 rs3753841 genotype and elbow tendon pathology, as explained in the following section, a one-way ANOVA was used to find differences among the three genotypes and elbow range of movement: flexion, extension, pronation and supination for both arms. A statistically significant difference was found in the flexion of the right elbow (p=0.005), with the CC genotype having greater mean flexion range than the CT and TT genotypes (141 vs. 137 and 137 degrees flexion, respectively).

3. Genetic factors

3.1. COL5A1 rs12722

Allele frequency for this SNP was 65.69% cytosine/34.31% thiamine for the first allele, and 8.76% cytosine/91.24% thiamine for the second allele. The genotype distribution was 8.76% CC, 56.93% CT, 34.31% TT (Figure 23).

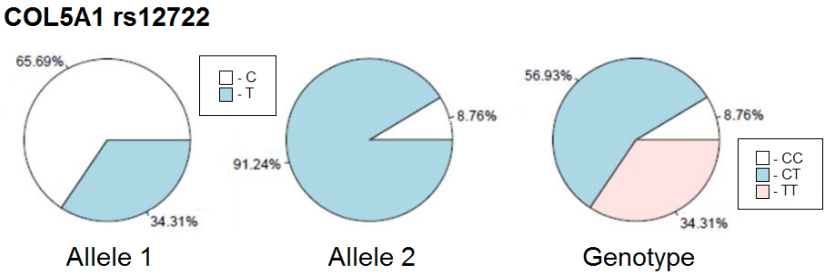

Figure 23. Allele and genotype frequency for COL5A1 SNP rs12722.

This particular SNP has been correlated with tennis elbow in a previous study (Altinisik et al., 2015); however, upon analysis of our data with a chi-squared test, no significant difference was found between the normal and tendinopathy groups with respect to COL5A1 genotype. Further analysis using self-organized maps does reveal a tendency towards an association, however, as shown in section 6.1 "Self-organizing maps".

3.2. COL11A1 rs1676486

Allele frequency for this SNP was 77.37% cytosine/22.63% thiamine for the first allele, and 97.08% cytosine/2.92% thiamine for the second allele. The genotype distribution was 77.37% CC, 19.71% CT, 2.92% TT (Figure 24). Chi-squared analysis did not show a significant difference between normal and tendinopathy groups with respect to the COL11A1 rs1676486 SNP genotype.

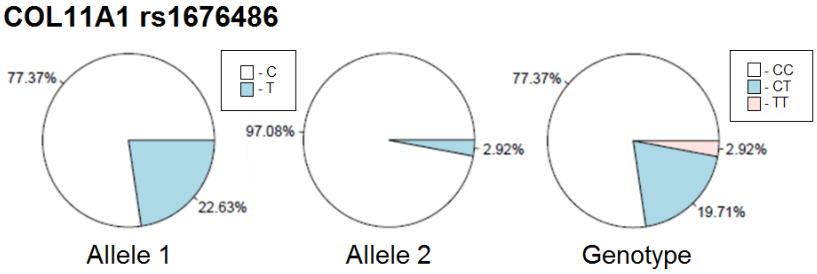

Figure 24. Allele and genotype frequency for COL11A1 SNP rs1676486.

3.3. COL11A1 rs3753841

Allele frequency for this SNP was 65.69% cytosine/34.31% thiamine for the first allele, and 16.79% cytosine/83.21% thiamine for the second allele. The genotype distribution was 16.79% CC, 48.91% CT, 34.31% TT (Figure 25).

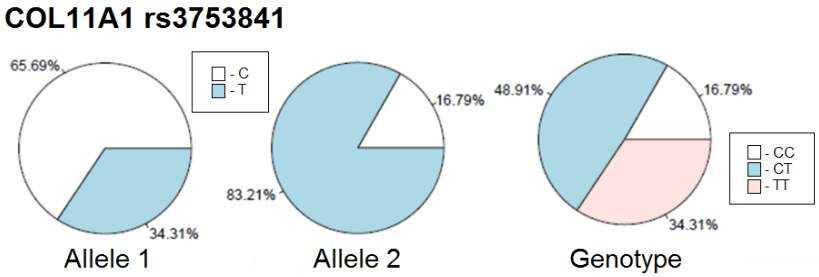

Figure 25. Allele and genotype frequency for COL11A1 SNP rs3753841.

Through the chi-squared test, we found a significant association between this SNP's genotype and the presence of tendinopathy in the elbow (p=0.025). Participants with genotype CT

had tendinopathy with greater frequency than the two homozygous genotypes (Figure 26).

This SNP also had a significant relationship to the other COL11A1 SNP studied, COL11A1 rs1676486 ($p=6.04 \times 10^{-8}$), despite the fact that this other SNP was not associated with elbow tendon pathology.

Finally, there was a significant association between this SNP and participants reporting a family history of high cholesterol ($p=0.027$).

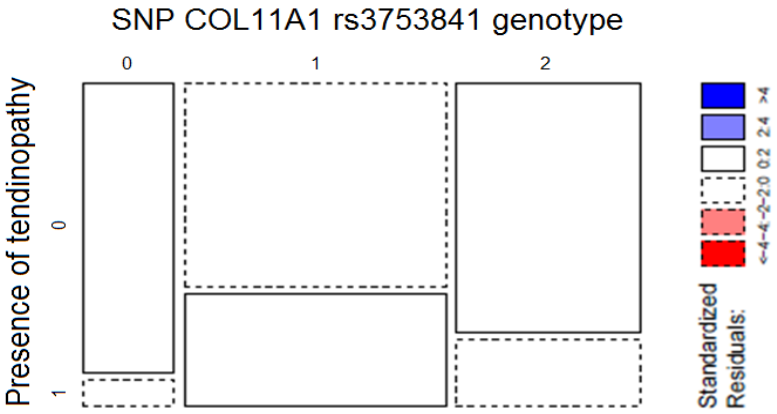

Figure 26. Mosaic for SNP COL11A1 rs3753841 genotype (x axis) and elbow tendinopathy (y axis). Genotype: 0 = CC, 1 = CT, 2 = TT. Tendinopathy: 0 = absence, 1 = presence of tendinopathy.

3.4. COL11A2 rs1799907

Allele frequency for this SNP was 37.23% adenosine/62.77% thiamine for the first allele, and 9.49% adenosine/90.51% thiamine for the second allele. The genotype distribution was 9.49% AA, 27.74% AT, 62.77% TT (Figure 27).

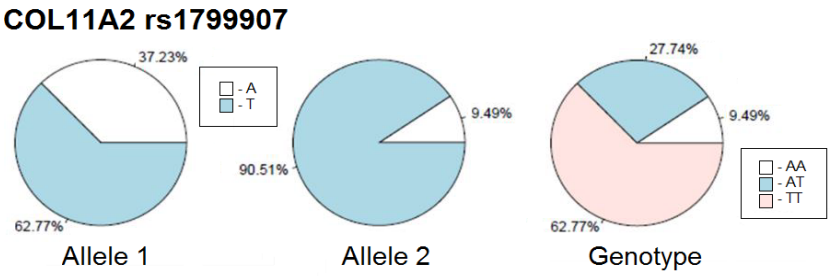

Figure 27. Allele and genotype frequency for COL11A2 SNP rs1799907.

Chi-squared analysis did not show a significant difference between normal and tendinopathy groups with respect to the COL11A2 rs1799907 SNP genotype.

Genotype frequencies for all SNPs studied in the case and control groups, as well as results of the chi-squared associations, are summarized in Table 3.

Table 3. Genotype frequencies for SNPs studied

	Cases (n=36)	Controls (n=101)	p
Genotype COL5a1 rs12722, n (%)			0.844
C/C	4 (11.1)	8 (7.9)	
C/T	20 (55.6)	58 (57.4)	
T/T	12 (33.3)	35 (34.7)	
Genotype COL11a1 rs3753841, n (%)			0.024*
C/C	2 (5.6)	21 (20.8)	
C/T	24 (66.7)	43 (42.6)	
T/T	10 (27.8)	37 (36.6)	
Genotype COL11a1 rs1676486, n (%)			0.862
C/C	29 (80.6)	77 (76.2)	
C/T	6 (16.7)	21 (20.8)	
T/T	1 (2.8)	3 (3.0)	
Genotype COL11a2 rs1799907, n (%)			0.807
A/A	4 (11.1)	9 (8.9)	
A/T	11 (30.6)	27 (26.7)	
T/T	21 (58.3)	65 (64.4)	

C = cytosine, T = thymine, A = adenine; *significant, $p<0.05$

4. Other intrinsic risk factors

Other intrinsic factors were significantly associated with the presence of elbow tendinopathy. Most notably, these variables included higher values for BMI (p=0.00002) and related variables, such as percent body fat (p=0.00003), weight (p=0.001), and waist circumference (p=0.003) (Figure 28). There were no significant differences between the two groups in age or gender composition.

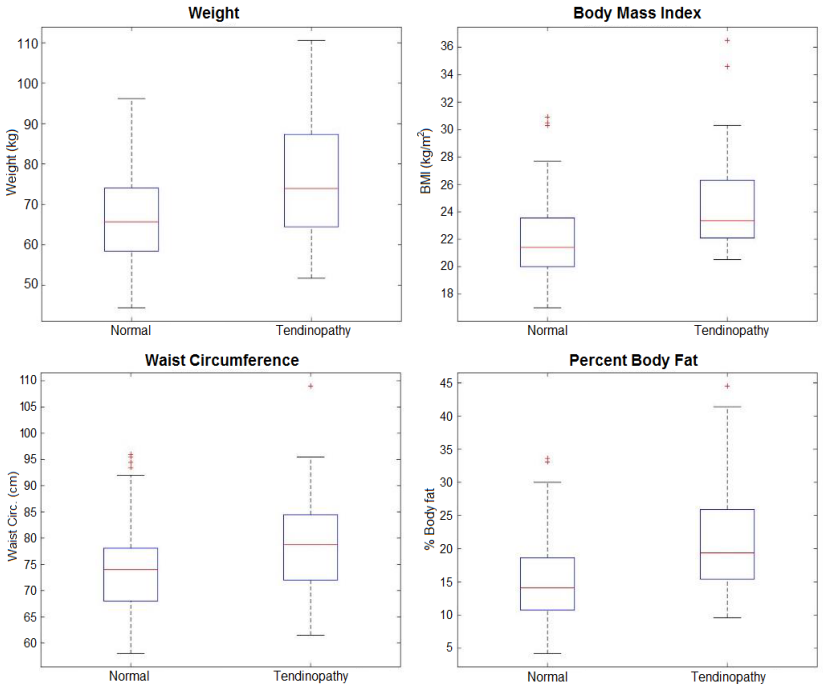

Figure 28. Significant differences in weight, BMI, waist circumference, and percent body fat between the normal and tendinopathy groups, with the tendinopathy group having higher values for all four factors.

5. Logistic regression

A logistic regression was also used to control for certain intrinsic variables that have been reported to have an association with tendon pathology, such as age and BMI, as well as sex, in order to account for potentially confounding effects of these variables when determining a possible association between SNP genotypes and pathology. In the logistic regression model, the relationship between the COL11A1 rs3753841 SNP genotype ceased to be significant when controlling for BMI.

6. Machine learning

6.1. Self-organized maps

Self-organized maps (SOMs) were created from normalized data in order to jointly visualize the different variables of interest and determine their relationship to the presence of tendinopathy and amongst each other. Figure 29 shows the SOM for genotypes of the three SNPs which showed a correlation with tendinopathy.

The clustering of related colors on the SOM in the top row indicate the relationship between these variables and the presence of tendinopathy. However, this relationship can be understood as merely a guideline since tendinopathy cases are not found exclusively in this row.

Further analysis using SOMs revealed relationships between other variables as well, as shown in Figure 30. Factors which were analyzed but did not reveal any apparent tendencies with relationship to elbow tendinopathy included age, smoking status, handedness, and family history of diabetes.

Some variables were excluded from SOM analysis due to their dependency on other variables (e.g. waist circumference, strongly correlated with weight and BMI; mobility of left elbow, strongly

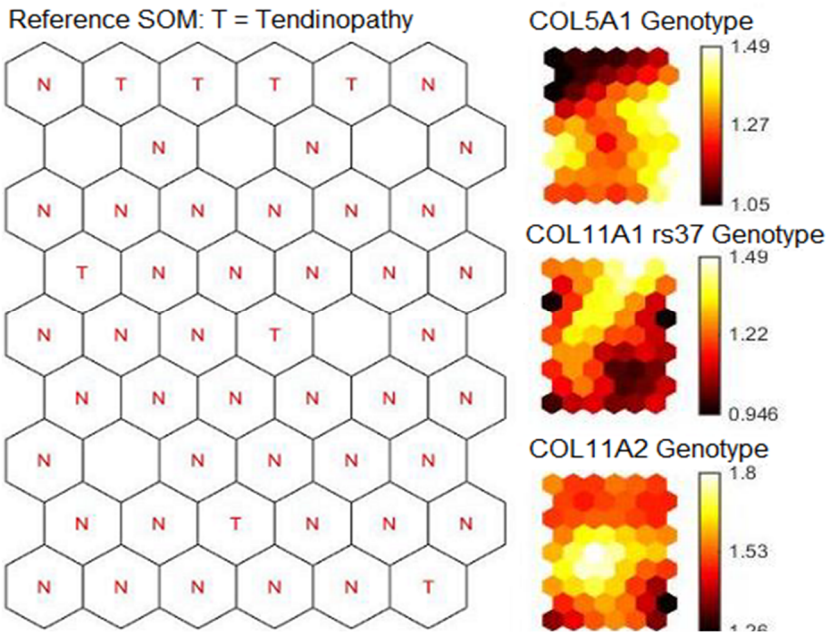

Figure 29. Self-organized maps of presence/absence (T/N) of tendinopathy and genotypes for SNPs COL5A1 rs12722, COL11A1 rs3753841, and COL11A2 rs1799907.

correlated with mobility of right elbow) according to the Pearson correlation (correlation ≥0.80).

Aside from the apparent relationships between these factors and elbow tendinopathy, we can also infer relationships between the factors themselves. For example, a higher percent body fat is correlated with increased laxity and hypermobility, as well as lower levels of shoulder activity.

6.2. Random forest

A random forest test was used to assess the relative importance of each tested variable, having performed better compared to the linear, discriminant linear, and decision tree tests (training = 1, validation = 0.64); results are shown in Figure 31. The variables with the strongest prediction of belonging to the normal or tendinopathy group were: percent body fat, BMI, shoulder activity level, and height.

Among genetic factors, the COL11A1 rs3753841 SNP genotype had the greatest influence, followed by COL11A2 rs1799907 genotype and COL5A1 rs12722 genotype, although the latter two were not much more influential than the least important factors according to this test (smoking status, handedness/laterality, family history of diabetes and right elbow instability).

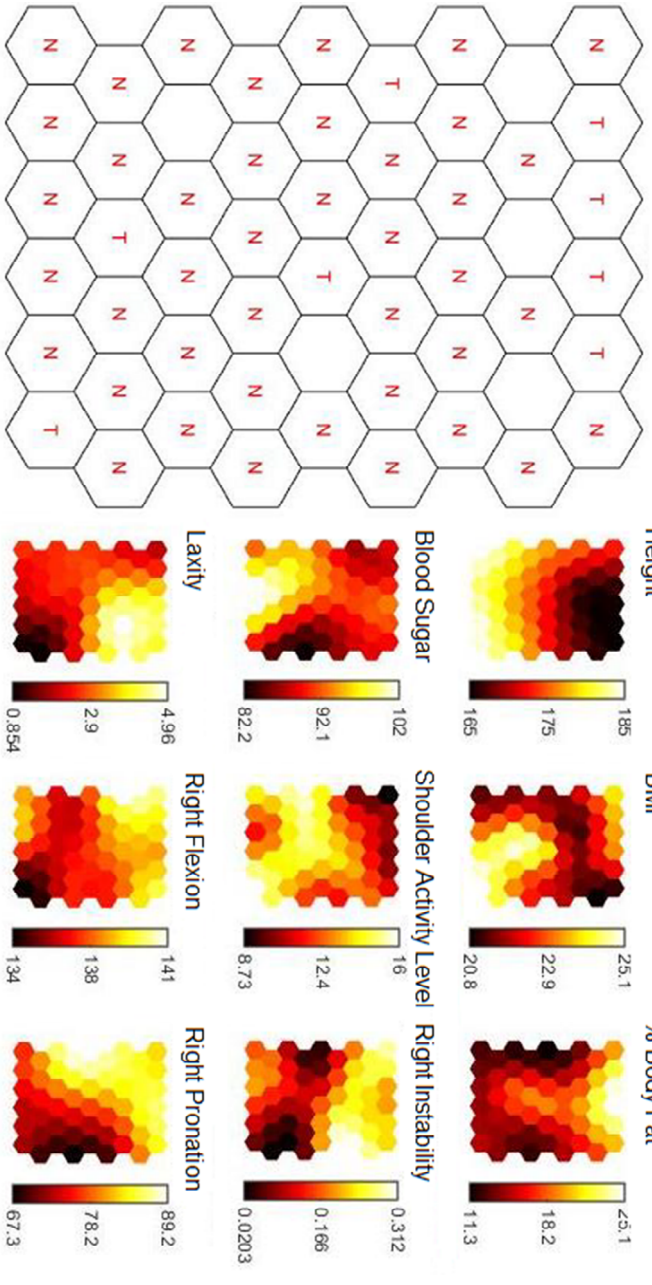

Figure 30. Self-organized maps of presence/absence (T/N) of tendinopathy and biological factors contributing to elbow tendinopathy: height, BMI, % body fat, blood sugar, shoulder activity level, right elbow instability, laxity, right elbow flexion, right elbow pronation.

Of intermediate importance were measures of joint hypermobility (right elbow supination, flexion, and pronation), age, blood sugar, and overall laxity as measured by the Beighton test.

Based on the results of the random forest test, the COL11A1 rs3753841 SNP genotype had the greatest influence of the genetic variants studied, followed by COL11A2 rs1799907 genotype and COL5A1 rs12722 genotype. Anthropometric factors were generally found to be more statistically important than genetic factors.

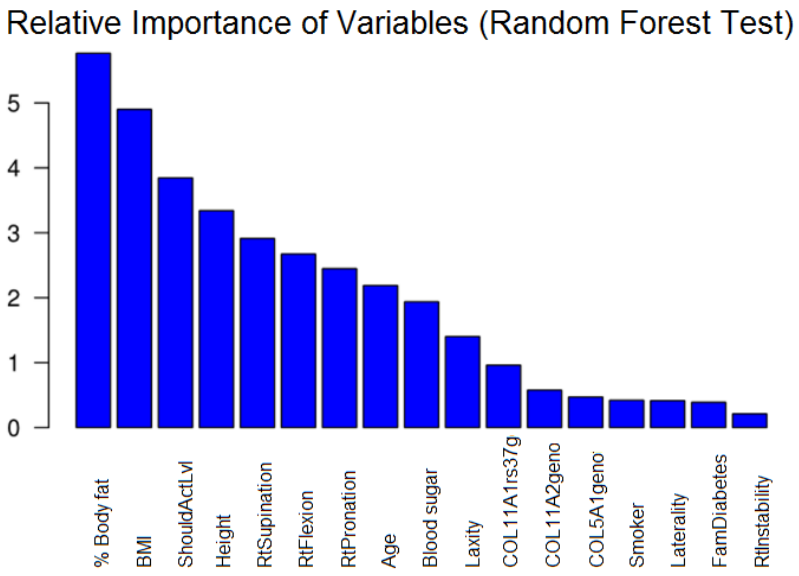

Figure 31. Random forest test showing the relative importance of tested variables.

IV. DISCUSSION

The objective of the present work was to identify and describe certain risk factors that influence the development of elbow tendon pathology. Primarily, the study focused on the relationship between specific genetic polymorphisms and the presence or absence of elbow tendinopathy. In addition, demographic, anthropometric and lifestyle data were collected in order to discover other intrinsic and extrinsic risk factors. Finally, a machine learning analysis was used to determine the degree to which each risk factor affects this pathology. Besides analyses used to find differences between the case and control groups, further analyses were performed in some cases to look for differences within an individual group, usually stratified by genotype for the SNPs studied.

The results of these various analyses are discussed below, beginning with the significant differences in DASH scores between the two groups, followed by sports participation and functional tests, genetic factors, and metabolic risk factors.

1. Diagnostic criteria and DASH scores

The tendon pathology group as determined by abnormal tendon structure on imaging had DASH sports and occupational scores that were significantly higher than those of the normal group ($p=0.03$ and $p=0.005$, respectively), reflective of higher perceived pain and lower perceived functionality. We believe that this significant

difference supports our use of structural abnormalities upon US imaging as our diagnostic criteria for assigning participants to the pathological or normal groups.

It is typical for symptomology to be used as diagnostic and/or group allocation criteria in tendinopathy studies; however, it is becoming clear that symptoms are variably associated with pathology, making it a less reliable indicator of an underlying tendon structure problem, which could result in a substantial number of study participants with structural tendon pathology being sorted into control groups based on their lack of symptoms.

The significance of DASH scores in predicting the presence or absence of tendinopathy in the elbow is reasonable considering findings by other authors regarding the inability to satisfactorily perform athletic and work-related activities with the upper limb when suffering from a degenerative tendon condition (Vicente-Herrero et al., 2009; Edmonds & Dengerink, 2014).

The DASH data also offer reassurance that false negatives (i.e. normal tendon structure on imaging but with symptoms characteristic of elbow tendinopathy) were not a meaningful issue in this study. While this questionnaire does not have threshold scores to separate participants into groups (e.g. functional/dysfunctional; mild/moderate/severe, etc.), normative values for the general

population have been published by the American Academy of Orthopedic Surgeons (Hunsaker, 2002). The mean published in that study was a score of 10.1 on the DASH General questionnaire, with a standard deviation of 14.68. We are confident that false negatives were not a problem because more than 99% of our control group participants had scores which fell easily into this normal range.

2. Sports participation and functional tests

We found a significant relationship between participation in throwing sports, as opposed to contact sports, and belonging to the elbow tendinopathy group. Among throwing athletes, subjects who played competitively but not professionally were the most likely to suffer from elbow tendinopathy. This is in agreement with previous studies that have reported amateur athletes as being more likely to suffer tendinopathy than professionals (Mishra et al., 2014), presumably because those playing at the elite or professional level benefit from superior training, technique and sports medicine care.

As for contact sports, no significant relationship was found with the presence of elbow tendinopathy. However, we did find a relationship between participation in contact sports and instability in both the right and left elbow joint. While this does not necessarily indicate that playing contact sports is the cause of increased elbow instability, it is an interesting association that athletes and sports

clinicians may want to consider. There was also a significant relationship between sex and instability of both elbows, with women being more likely to present elbow instability; as in the previous case, female athletes and their physiotherapists should take this into account.

We did not find a significant relationship between joint laxity and tendinopathy. However, a one-way ANOVA did reveal significant differences in laxity among the three genotypes for one of the SNPS studied, COL5A1 rs12722. The CT genotype had a higher mean laxity than the CC and TT genotypes.

Upon discovering the significant relationship between SNP COL11A1 rs3753841 genotype and elbow tendon pathology, as explained in the following section, a one-way ANOVA was used to find differences among the three genotypes and elbow range of movement: flexion, extension, pronation and supination for both arms. A statistically significant difference was found in the flexion of the right elbow ($p=0.005$), with the CC genotype having greater mean flexion range than the CT and TT genotypes (141 vs. 137 and 137 degrees, respectively).

3. Genotype relationships and distributions

Genotype distributions for COL5A1 rs12722 were different from those found in previous studies, with apparent ethnic factors

involved in these differences. A study by Kim et al, (2015) on Korean ballerinas found a genotype distribution for this SNP of: 53.8% CC, 40.7% CT, 4% TT. Similarly, a study done in a Japanese population had the following results: 76% CC, 24% CT + TT (Kubo et al., 2013). On the other hand, a 2014 study by Bertuzzi et al. in a Brazilian population reported 16% CC, 56% CT and 28% TT. In our Spanish population, the values were 8.76% CC, 56.93% CT, and 34.31% TT.

Genotype distributions for COL11A1 rs1676486 are comparable to those obtained by Hay et al. (2013) in a study on a combined Caucasian South African/Australian population of European ancestry. In their study, genotype distribution in the entire study population for this SNP was: 66.3% CC, 28.9% CT, and 4.8% TT, compared to our values of 77.37% CC, 19.71% CT, and 2.92% TT.

Genotype distributions for COL11A1 rs3753841 are also comparable to those obtained by Hay et al. (2013). In their study, genotype distribution in the entire study population for this SNP was: 16.4% CC, 44.4% CT, 39.2% TT. This compares to our values of 16.79% CC, 48.91% CT, and 34.31% TT.

Genotype distributions for COL11A2 rs1799907, however, were quite different from those obtained by Hay et al. (2013). In their study, genotype distribution in the entire study population for this SNP

was: 43.5% AA, 43.3% AT, 13.2% TT, compared to the values in our study of 9.49% AA, 27.74% AT, 62.77% TT. Despite both study populations being made up of European subjects, this could be due to genetic differences between British Isles and Southern Europe populations, but further study in other populations would be needed to confirm this.

The only SNP which we found to have a statistically significant relationship with elbow tendinopathy was COL11A1 rs3753841; however, we can also see a tendency towards a relationship for the COL11A2 and COL5A1 SNPs in the SOMs. These results corroborate those of other studies investigating genetic factors influencing other tendinopathies, namely the shoulder, knee and Achilles tendon (Jelinsky et al., 2011; Mokone & Schwellnus, 2006).

COL5A1 rs12722 has been associated with tennis elbow in a previous study (Altinisik et al., 2015). However, we found no significant relationship with respect to this genotype. This could be due to the fact that our study considered both medial and lateral elbow tendons. Our diagnostic criteria were also different, as Altinisik et al. relied on clinical criteria that did not involve imaging through ultrasound or any other means. Imaging is the only method to confirm tendon pathology as symptoms are variably associated with pathology (Rio et al., 2014).

To our knowledge, this is the first reporting of COL11A1 rs3753841 genotype as a risk factor for elbow tendon pathology. Additional support for this relationship despite a sample size that is smaller than the ideal is the fact that the genotype distributions for this SNP are within the Hardy-Weinberg Equilibrium (HWE) expected values ($\chi 2 = 0.01$). Furthermore, the power of our study with the sample size used is reasonably strong; the power of the chi-square tests used in the study to detect a significant relationship between genotype and elbow tendon pathology, with a medium effect size and significance level of 0.05, is 89.20%.

3.1. Genetic mechanism

The genetic mechanism by which the CT genotype of COL11A1 rs3753841 is not immediately clear. Two common explanations for SNP variants influencing a pathology phenotype can largely be ruled out.

First, the reference genotype for this SNP, the CC variant, leads to the collagen XI α-1 chain having a proline at that position. This proline is substituted by a leucine when the T allele is present at this SNP location. On the one hand, this type of amino acid substitution is not likely to cause a change in the protein chain's structure or stability to the point that it would have pathological consequences. And, on the other hand, if the increased risk of tendon pathology were due to a

compromised α-1 chain, we would expect the TT genotype to be the risk variant, rather than the heterozygous genotype.

Secondly, SNP's sometimes cause phenotype changes because of how they influence levels of gene expression in the cell. Epigenetic mechanisms of increased tendinopathy risk have been studied lately, but this is also not a likely explanation for the relationship between COL11A1 rs3753841 and elbow tendon pathology. This is because this SNP is not located near a regulatory site on the gene in question. If it were, we might speculate that the substitution of a C allele for a T was somehow causing a change in how the gene's expression is regulated, perhaps because of a structural difference as in the case of DNA methylation. However, since the SNP is so distant from the gene's promoter region, and also because it would be unusual for the heterozygous genotype to be the risk variant if this were the mechanism, it seems an unlikely explanation for why participants in the tendon pathology group were significantly more likely to have the CT genotype for this particular SNP.

4. Metabolic factors and tendinopathy

The significant impact of anthropometric factors such as percent body fat and BMI on the presence of tendinopathy is, initially, not surprising considering the amount of literature available relating obesity to degenerative tendon conditions (Gaida et al., 2009;

Franceschi et al., 2014). Despite the number of studies linking a high BMI with risk of tendon pathology, however, the exact mechanism to explain this relationship remains unclear.

In the 2014 review by Franceschi et al., elevated BMI was closely related with increased risk of tendinopathy in the lower limb. Given our understanding of how the tendon responds to loading, it is logical that tendon degeneration would increase in, for example, the Achilles tendon, in an overweight person due to being subjected to an excessive load. Another epidemiological study on 5000 patients with rotator cuff tendinopathy found that a BMI greater than 25.1 was associated with this pathology (Titchener et al., 2014). Similarly, a study by Gumina et al. (2014) reported elevated BMI as a risk factor in tendon rupture in the rotator cuff. However, a more recent study by Applegate et al. (2017) on the influence of cardiovascular disease (CVD) risk factors on shoulder disorders did not find a significant difference in BMI between rotator cuff tendinopathy patients and controls in a population of adults employed in production facilities in a combination of low, moderate and highly physically demanding jobs. Of all the CVD risk factors studied, they only found a significant relationship for two: hypertension and systolic blood pressure.

Several studies have proposed that obesity leads to increased risk of musculoskeletal (MSK) disorders due to the chronic, low-grade

inflammation that is produced by visceral fat. This inflammation leads to dysregulated tissue repair which, in turn, increases the risk of osteoporosis and tendinopathy (Collins et al., 2018). One known mechanism linking adiposity to tendon dysrepair is via proinflammatory cytokines, such as IL-1b, TNF-a, and IL-6, which is increased in the adipose tissue of obese animals. These cytokines can then induce ECM degradation, induce other pro-inflammatory cytokines, and affect collagen and elastin expression (Collins et al., 2018). Another proposed mechanism is adipokine modulation of the production of certain enzymes which are important in the functioning of tenocytes (Kozlovskaia et al., 2017). Finally, it is logical that a sedentary lifestyle, frequently associated with obesity, would be unfavorable for tendon health due to insufficient loading for collagen synthesis and repair.

In fact, due to the previously reported relationship between BMI and tendinopathy in tendons other than the elbow, we felt it was appropriate to perform a logistic regression in order to control for potentially confounding effects of this association on any relationships found between genetic factors and pathology. Interestingly, in our logistic regression model controlling for BMI, the relationship between the COL11A1 rs3753841 genotype and elbow tendon pathology was no longer statistically significant. A possible explanation for this would be a BMI-genotype interaction which leads to increased risk.

However, the values for BMI and related metabolic factors in our study are generally lower than in other studies because our study population is composed of younger athletes, who are less likely to have a high BMI and percent body fat. For this reason, the fact that such a significant relationship exists even in our population is a very relevant and interesting finding.

Furthermore, given that the elbow is an area of the body with very little adipose tissue locally, this seems to point to a systemic mechanism, as opposed to an effect of locally-produced inflammation resulting from adipose tissue around the tendon, as has been previously suggested (Masiero et al., 2018), although the fat pad located at the elbow joint could be a source of adipokines. Another interesting consideration is that, unlike other tendons, the elbow is not as heavily affected by a person being overweight or obese. In the case of the Achilles tendon, the knee, and even the rotator cuff, it could be argued that there is an increased risk of tendon pathology simply because of an excessive load on that tendon. But loading of the elbow tendon would, in most situations, be determined by the weight of the forearm, wrist, hand, and any external objects. Even in people who are obese, the amount of extra weight stored at that part of the body is low, and in our population of young, healthy athletes, it seems especially unlikely that this could be attributed to increased loading alone.

As for other metabolic factors studied in the present work, no significant relationships were found. However, it is difficult for us to make an apples to apples comparison with previous studies because we did not measure each participant's blood lipid panel; rather, they were asked if they had a family history of high cholesterol, diabetes, and hypertension. In the literature, there is conflicting evidence on the influence of hypertension, high cholesterol, and diabetes mellitus on risk of tendinopathy. In their study of CVD risk factors and rotator cuff tendinopathy, Applegate et al. (2017) only found a statistically significant relationship with systolic blood pressure and hypertension. Tilley et al. (2015) found an association between high cholesterol and tissue damage in tendons. It is possible that we would have found these relationships as well had we done a complete blood analysis for each participant.,

5. History of previous tendinopathy

Given the multiple and diverse intrinsic factors which influence an individual's predisposition for elbow tendinopathy and other tendinopathies, it is not surprising that a history of previous tendinopathy was significantly associated with current elbow tendinopathy; many of the extrinsic and intrinsic factors which led to the first degenerative tendon condition would reasonably be expected to lead to another case in the elbow for subjects who routinely load the

elbow tendons or perform repetitive movements with the muscles attaching to these tendons.

6. Machine learning models

The self-organized maps (SOMs) used in the data analysis of the present study are a useful tool for visualizing a complex, multifactorial condition like elbow tendinopathy, as it allows us to simultaneously analyze numerous variables, both quantitative and categorical, with a simple graphic in two dimensions (Sotolongo et al., 2002). We can therefore use this tool to construct a visual map of the behavior of the variables that affect a given pathology, in this case, tendinopathy of the elbow, and thus stratify large samples of subjects according to those variables.

Likewise, the use of selection models like the random forest test provide valuable information that allow clinicians to be more discerning in the variables they assess, being able to select those variables which are more influential in the development of elbow tendinopathy or other pathologies.

7. Limitations of the study

The primary limitations of this study involve our inability to directly collect certain metabolic data, such as blood lipid profile, relying instead solely on the family history reported by the subjects. It

is possible that this was overlooked as a significant risk factor because of the limitations of the data.

Another limitation of this study has been the sample size resulting from financial limitations given the high cost of genetic testing, although similar sample sizes have been used by other authors (Collins et al., 2009; Bertuzzi et al., 2014; Salles et al, 2015).

8. Future research

Firstly, there is the fact that a significant association was found between elbow tendon pathology and several related anthropometric measurements, namely BMI, percent body fat and waist circumference. A logistic regression returned a non-significant relationship between the SNP of interest and pathology when controlling for BMI. However, an alternative and very interesting explanation is a possible BMI-genotype interaction whereby having a higher BMI causes increased risk of elbow tendon pathology in individuals with the CT genotype more than in other genotypes.

In the future, it could be interesting to investigate the relationship of these variables in greater depth, since there is also a possibility that a BMI-genotype interaction is at play here. Such an interaction could offer insight into the genetic mechanism by which the COL11A1 3753841 CT genotype is a risk factor for elbow tendon pathology, since reduced structural integrity of the α1 chain does not

seem to be a likely cause, and this SNP is far enough away from the promoter region that a simple change in gene regulation is not a suitable explanation, either.

In summary, the present study presents new potential lines of research, including a more complete analysis of genetic factors related to collagen regulation and other components of the tendon extracellular matrix and a deeper investigation into the mechanism by which BMI may increase risk of not only elbow tendinopathy, but other tendinopathies as well.

The results of this study also offer several practical implications which could be taken into account by clinicians. For one, participants with elbow tendons classified as pathological using ultrasound imaging reported significantly higher levels of elbow pain and dysfunction; this serves as additional support in the use of ultrasound imaging as a reliable method to diagnose tendon pathology, even when symptoms are ambiguous. Also, a significant relationship was found between the COL11A1 rs3753841 genotype and structural abnormality in elbow tendons; although currently the cost of genetic testing may make genetic stratification unfeasible for many clinicians, this could be a useful tool as costs continue to decline or in patients with recurrent episodes of elbow tendinopathy that are not easily explained by other factors. Finally, a significant relationship was also found between structural abnormality in elbow tendons and BMI, as well as percent body fat and waist circumference. This is additional

evidence to support clinicians encouraging their overweight patients to lose weight in order to reduce tendinopathy risk. In particular, those with the risk variant of COL11A1 rs3753841 who are overweight should be encouraged to lose weight, as there may be a BMI-genotype interaction.

V. CONCLUSIONS

The etiology of elbow tendinopathy is multifactorial, given that we have found many diverse intrinsic factors which influence its presence. These include genetic factors as well as anthropometric measurements and functionality scores:

1. The genotype for SNP COL11A1 rs3753841 was associated with incidence of elbow tendinopathy (p=0.025); subjects in the tendinopathy group were significantly more likely to have the CT genotype. None of the other SNPs studied showed a significant association; however, there is a tendency towards a relationship for the COL11A2 rs1799907 and COL5A1 rs12722, as shown in the self-organized maps.

2. Several other intrinsic factors were significantly associated with the presence of elbow tendinopathy, including: higher values for weight (p=0.001), BMI (p=0.00002), waist circumference (p=0.003), percent body fat (p=0.00003), and DASH scores for the sports (p=0.03) and occupational (p=0.005) sections, and previous incidence of tendinopathy in either the right side or bilaterally (p=0.02). Other factors showed a tendency toward an association, such as: shorter stature, elevated blood sugar, right elbow hypermobility, and laxity.

3. Participation in throwing sports was associated with elbow tendon pathology (amateur competitive, p=0.03). Contact sports were not significantly associated with the pathology, but a relationship was found between participation in these sports and instability in both elbows.

4. Based on the results of the random forest test, the most influential factors of those studied were anthropometric: percent body fat, BMI, shoulder activity level, and height. Genetic factors were found to be less statistically important variables than elbow hypermobility, age, blood sugar, and laxity, but more important than smoking status, handedness/laterality, family history of diabetes, and, surprisingly, elbow instability. The genetic factors, in order of decreasing importance, were: COL11A1 rs3753841 genotype (statistically significant), COL11A2 rs1799907 and COL5A1 rs12722 (neither of which was statistically significant).

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VII. APPENDICES

Appendix 1: Ethics committee authorization

VNIVERSITAT DE VALÈNCIA Vicerectorat d'Investigació i Política Científica

D. Fernando A. Verdú Pascual, Profesor Titular de Medicina Legal y Forense, y Secretario del Comité Ético de Investigación en Humanos de la Comisión de Ética en Investigación Experimental de la Universitat de València,

CERTIFICA:

Que el Comité Ético de Investigación en Humanos, en la reunión celebrada el día 20 de noviembre de 2014, una vez estudiado el proyecto de investigación titulado: *"Análisis de los marcadores genéticos y otros factores de riesgo asociados a la tendinopatía del miembro superior", número de procedimiento H1409657453224,* cuyo investigador responsable es D. Yasser Alakhdar Mohmara,
ha acordado informar favorablemente el mismo dado que se respetan los principios fundamentales establecidos en la Declaración de Helsinki, en el Convenio del Consejo de Europa relativo a los derechos humanos y cumple los requisitos establecidos en la legislación española en el ámbito de la investigación biomédica, la protección de datos de carácter personal y la bioética.

Y para que conste, se firma el presente certificado en Valencia, a veintisiete de noviembre de dos mil catorce.

FERNANDO ALEJO|VERDU|PASCUAL
2014.12.01
08:27:32 +01'00'

Appendix 2: Informed consent form

1.- Identificación y descripción del procedimiento.

"Estudio del comportamiento genético y valoración física en las tendinopatias".

El procedimiento que se le propone consistirá en la realización de un/unos análisis genético/s a partir de una muestra de sangre para detectar la presencia, ausencia o variantes de uno o varios segmentos de material genético, pudiendo incluir pruebas indirectas para la detección de productos génicos o metabolitos específicos indicativos de cambios genéticos determinados y una valoración física mediante test de movilidad y estudio de ecografía.

2.- Objetivo.
La finalidad de todos los análisis que se le proponen, así como aquellos que se le pudieran hacer en un futuro es, sobre todo, detectar posibles mutaciones, poder analizar el riesgo de padecer una tendinopatía y proceder a la correcta caracterización / diagnóstico de las tendinopatías y la optimización del manejo clínico de la misma.
Debe saber, en cualquier caso, que se le informará verbalmente de los resultados de los mismos.
Las muestras destinadas al análisis genético, incluyendo las pruebas indirectas para la detección de productos génicos o metabolitos específicos indicativos de cambios genéticos determinados, se realizarán en los distintos laboratorios acreditados para tal fin de la institución que está colaborando en este estudio: anatomía patológica, análisis clínicos, hematología, microbiología, genética y biología molecular.
Las muestras, una vez procesadas, se almacenarán en la facultad de Medicina, laboratorios de la Universidad de Valencia durante el tiempo necesario para realizar todo el proceso de análisis descrito y a continuación serán destruidas, salvo que se estime la conveniencia de otros usos para lo que se requerirá, nuevamente, su consentimiento.
En el caso en el que los análisis genéticos se deban hacer fuera de la Institución que le está prestando asistencia, sus datos de identificación personales serán debidamente codificados.

3.- Beneficios esperados.
Los resultados del análisis genético se evaluarán teniendo en cuenta los antecedentes clínicos personales y familiares, los resultados de la exploración física, las pruebas complementarias y la interpretación clínica por el personal facultativo. En todo momento será debidamente informado de las repercusiones que los análisis genéticos y físicos vayan a tener sobre el manejo clínico de su patología si la hubiera.

4.- Consecuencias previsibles de su realización.
Es posible que los estudios realizados sobre sus muestras aporten información relevante para su salud o la de sus familiares. Tiene derecho tanto a ser informado como a que no se le informe de sus datos genéticos y otros datos personales obtenidos en el estudio. A estos efectos, se entenderá que desea recibir tal información salvo que manifieste lo contrario.
Estos datos pueden repercutir en algunos miembros de su familia, por lo cual usted valorará la conveniencia de transmitirles dicha información.

5.- Consecuencias previsibles de su no realización y derecho de revocación del consentimiento.
La decisión de no realizarse el estudio genético es totalmente voluntaria, pudiendo negarse e incluso pudiendo revocar su consentimiento en cualquier momento, sin tener que dar ninguna explicación y sin que ello tenga ninguna repercusión de ningún tipo.

6.- Protección de datos personales y confidencialidad.
Los datos resultantes de los análisis se almacenarán en el archivo de la unidad.
Los profesionales sanitarios del centro tendrán acceso a los datos que consten en su historia clínica en tanto sea pertinente para la asistencia que le presten. El personal que acceda a los

datos genéticos en el ejercicio de sus funciones quedará sujeto al deber de secreto de forma permanente.
Ha de saber que la información sobre sus datos personales y de salud serán incorporados y tratados en una base de datos informatizada cumpliendo con las garantías que establece la Ley de Protección de Datos de Carácter Personal y la legislación sanitaria aplicable.
Los datos genéticos de carácter personal se conservarán durante un periodo mínimo de 5 años, tras los cuales podrá solicitar su cancelación. Para solicitar la cancelación deberá hacerlo por escrito y dirigirse a los profesionales del centro que le propuso la colaboración. En caso de que usted no solicitara dicha cancelación, los datos se mantendrán indefinidamente.

Declaración del paciente: D/Dña.....de.....años de edad, con domicilio en ..... . DNI .....
DECLARO Que el/la Dr./Dra....., me ha explicado el procedimiento a seguir para el estudio de genética y sangre. También se me ha informado de que podemos ser portadores de variantes genéticas que pueden predisponer al desarrollo de enfermedades tendinosas.
Manifiesto que estoy satisfecho/a con la información recibida, que se me ha informado verbalmente de los procedimientos de análisis genético y físico a los que voy a ser sometido, que he podido hacer las preguntas que he estimado conveniente, a las que se ha respondido adecuadamente, y que comprendo el alcance del procedimiento.

En Valencia a.....de.....de 20.....

Fdo:

Appendix 3: Data collection form

Sujeto:	

	Codo
Clasificación:	

Consentimiento:

Lado dominante:	

Glucemia:

Escala actividad hombro:

DASH:

Ecografía		
	Derecho	**Izquierdo**
Codo		
Epicondilo		
Epitróclea		

Custionario de genética:

Extracción:

Valoración ROM (º)			
Codo		**Derecho**	**Izquierdo**
	Flexión		
	Extensión		
	Abducción		
	Aducción		
Escala Beighton (0-1)			
	Quinto dedo		
	Pulgar		
	Codo		
	Rodilla		
	Flexión tronco		
	Total (sobre 9):		
	Mayor que 6 (+):		

Appendix 4: General questionnaire

A. Datos personales					
Apellidos					
Nombre					
Dirección			Código Postal		
E-mail			Teléfono		
Fecha de nacimiento	DD/MM/YYYY		Móvil		
Altura (cm)			Sexo	Hombre Mujer	
Peso (kg)		Circunferencia cintura (cm)		IMC	
Lesiones pasadas:			Actualmente:		
Ocupación:					
Ocupación antes de la lesión:					
Raza (Solo requerido y usada para propósitos científicos))	Negra/Africana Blanca India Asiática				
Ancestros	Padre		Desconocido		
	Madre		Desconocida		
País de nacimiento					

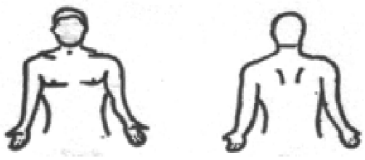
Mano dominante	Izquierda Derecha Ambidiestro
Pierna dominante	Izquierda Derecha Ambidiestro
Conoces tu grupo sanguíeno?	No Sí A neg A pos B neg B pos O neg O pos AB neg AB pos

Sección B. Detalles deportivos. **Por favor describa su participación en actividades deportivas.** **Use un formulario adicional si lo requiere.**			
Tipo de deporte(s) en los que ha participado, nómbrelos.	**Deporte principal 1**	**Otro deporte 2**	**Otro deporte 3**
Participación actual o pasada	Actual Pasado	Actual Pasado	Actual Pasado
Año de inicio			
Número de años de participación			
Años compitiendo			
Profesional o amateur			
Horas entrenamiento por semana (0-3 meses)			
Horas entrenamiento por semana (4-12 meses)			
Horas entrenamiento por semana (13-24 meses)			
Tipo de deporte(s) en los que ha participado, nómbrelos.	**Otro deporte 4**	**Otro deporte 5**	**Otro deporte 6**
Participación actual o pasada	Actual Pasado	Actual Pasado	Actual Pasado
Año de inicio			

Número de años de participación			
Años compitiendo			
Profesional o amateur			
Horas entrenamiento por semana (0-3 meses)			
Horas entrenamiento por semana (4-12 meses)			
Horas entrenamiento por semana (13-24 meses)			

Sección C. Estilo de vida y hábitos.			
Es fumador?	Fumador actual ☐	Ex fumador ☐	Nunca ☐
Si su respuesta es afirmativa, por favor conteste lo siguiente	Años fumando:	Si paró, ¿hace cuántos años?:	
	Numero de cigarros al día:		
De media, cuanto alcohol consume a la semana de vino, cerveza, cócteles.		_____ vasos de cerveza/semana _____ vasos de vino/semana _____ cócteles	
Historia familiar.			
Algún miembro de su familia padece de hipertensión o colesterol alto?	Si No		
Algún miembro de su familia padece diabetes?	Si No Tipo 1 Tipo 2		
Hay alguna historia familiar de Artritis?	Si No	En caso afirmativo, ¿qué tipo? Reumatoide Osteoartritis Otra	

Medicamentos	En caso afirmativo, ¿cuánto tiempo?	
Ha tomado alguna ver corticoesteroides orales (tabletas de Cortisona)	Si No ¿Cuántas veces?	3 meses 6 meses 12 meses 24 o más meses
Alguna vez le han inyectado corticoesteoides?	Si No ¿Cuántas veces?	3 meses 6 meses 12 meses 24 o más meses
Alguna vez le han inyectado corticoesteoides alrededor de un tendón?	Si No ¿Cuántas veces?	3 meses 6 meses 12 meses 24 o más meses
Alguna vez ha usado anabolizantes esteroideos?	Si No ¿Cuántas veces?	3 meses 6 meses 12 meses 24 o más meses
¿Alguna vez ha utilizado antibióticos fluoroquinolonas?	Si No ¿Cuántas veces?	3 meses 6 meses 12 meses 24 o más meses
En caso afirmativo, señale cuál		
Que médicamentos está tomando actualmente?		
Tiene alguna alergia?		

Sección D. Detalles médicos generales.		
Historia médica pasada		
Ha tenido alguna lesión tendinosa?	Si	No
En caso afirmativo, especifique cuál	Pie y tobillo:	Tendón de Aquiles
		Tibial posterior
		Fascia plantar
	Rodilla:	Tendón rotuliano
	Codo y muñeca:	Epicondíleos Epitrocleares Otros muñeca
	Hombro:	Subescapular
		Supraespinoso
		Infraespinoso
		Redondo menor
	Otras:_____	
Marque dónde se localiza su dolor:		
Ha tenido alguna lesión ligamentosa?	Sí	No
En caso afirmativo, especifique cuál	Lig. hombro	Lig. muñeca

	Lig, codo	Lig, dedos
	Rodilla (anterior)	Rodilla (posterior)
	Rodilla (interno)	Rodilla (externo)
	Lig. lateral externo tobillo	Lig. Lateral interno tobillo
	Lig. espinales	Otros

Sabe si alguien de su familia ha padecido lesiones tendinosas?	Sí No	En caso afirmativo especifique parentesco _____y tipo _____
Sabe si alguien de su familia ha padecido lesiones ligamentosas?	Sí No	En caso afirmativo especifique parentesco _____y tipo _____
Ha sufrido alguna de las siguentes patologías capsulares?	Luxación aguda de hombro Inestabilidad crónica Otras: _____	

Sufre algunas de las siguientes condiciones médicas?:

Hipertensión	Angina/infarto	Asma
Enfisema	Artritis reumatoide	Osteoartritis
Tumor maligno (cáncer)	Colesterol elevado	Desórdenes adrenérgicos
Patología de riñón	Diabetes mellitus	Desórdenes de tiroides
Amiloidosis.	En caso afirmativo, qué tipo? _____	

Sufre de alguna otra patología del tejido conectivo o reumatológica?	Sí No	

En caso afirmativo, señale a continuación:

Ha sido sometido a alguna operación? (Indique tipo y fecha)	
Si es mujer:	
¿A qué edad tuvo su primera menstruación? (años)	
¿Está tomando actualmente algún tipo de anticonceptivos?	Sí No
En caso afirmativo, ¿qué tipo?	Píldora Inyección DIU
Actualmente usted	Pre-menopáusica (±12 ciclos al año en intervalos de 23-33 días y sangrado de 3-7 días) Menopáusica (ciclos irregulares y menos frecuentes) Post-menopáusica (no presenta la menstruación) ¿Desde hace cuanto tiempo se encuentra en ésta última etapa?

Appendix 5: Shoulder activity level questionnaire

Por favor indique con una "x" con qué frecuencia es capaz de realizar las siguientes tareas en el momento que menos dolor haya experimentado y mayor actividad podía realizar, en el último año.

	Nunca o menos de una vez al mes	1 vez al mes	1 vez a la semana	Más de 1 vez a la semana	Diariamente
Transportar objetos de 3.5 Kg o más (como la bolsa de la compra)					
Llevar objetos por encima de la cabeza					
Elevar peso o entrenar pesas con los brazos					
Realizar movimientos oscilatorios (como golpear una pelota de tenis, de golf, beisbol u objetos similares).					
Elevar objetos de 11 Kg o más (como 3 garrafas de agua). No incluye levantamiento de peso.					

Para cada una de las siguientes cuestiones, por favor marque con un círculo la que mejor describa su participación en las actividades propuestas.

1) ¿Participa en algún deporte de contacto (como por ejemplo fútbol Americano, rugby, fútbol, baloncesto, boxeo, lacrosse, artes marciales,…)?

A .No

B. Sí, de manera recreativa.

C. Sí, deporte federado.

D. Sí, a nivel profesional.

2) Participa en algún deporte que involucre lanzamientos por encima de la cabeza (como beisbol, cricket, quarterback en fútbol Americano), saques por encima de la cabeza (como tenis o voleibol) o natación de distancia?.

A .No

B. Sí, de manera recreativa.

C. Sí, deporte federado.

D. Sí, a nivel profesional.

Appendix 6: DASH questionnaire

Cuestionario de Discapacidad del Brazo, Hombro y Mano (DASH*e*)

Califique su capacidad para realizar las siguientes actividades durante la última semana marcando con un círculo el número que figura bajo la respuesta correspondiente	Sin dificultad	Dificultad leve	Dificultad moderada	Dificultad severa	Incapaz
1. Abrir un bote apretado o nuevo	1	2	3	4	5
2. Escribir	1	2	3	4	5
3. Girar una llave	1	2	3	4	5
4. Preparar una comida	1	2	3	4	5
5. Empujar una puerta pesada para abrirla	1	2	3	4	5
6. Colocar un objeto en un estante por encima de la cabeza	1	2	3	4	5
7. Realizar tareas domésticas pesadas (p. ej., limpiar paredes o fregar suelos)	1	2	3	4	5
8. Cuidar plantas en el jardín o la terraza	1	2	3	4	5
9. Hacer una cama	1	2	3	4	5
10. Llevar una bolsa de la compra o una cartera	1	2	3	4	5
11. Llevar un objeto pesado (más de 5 kg)	1	2	3	4	5
12. Cambiar una bombilla que esté por encima de la cabeza	1	2	3	4	5
13. Lavarse o secarse el pelo	1	2	3	4	5
14. Lavarse la espalda	1	2	3	4	5
15. Ponerse un jersey	1	2	3	4	5
16. Usar un cuchillo para cortar alimentos	1	2	3	4	5
17. Actividades recreativas que requieren poco esfuerzo (p. e., jugar a las cartas, hacer punto)	1	2	3	4	5
18. Actividades recreativas en las que se realice alguna fuerza o se soporte algún impacto en el brazo, el hombro o la mano (p. ej., golf, tenis, dar martillazos)	1	2	3	4	5
19. Actividades recreativas en las que mueva libremente el brazo, el hombro o la mano (p. ej., jugar a ping-pong, lanzar una pelota)	1	2	3	4	5
20. Posibilidad de utilizar transportes (ir de un sitio a otro)	1	2	3	4	5
21. Actividades sexuales	1	2	3	4	5
22. Durante la semana pasada, ¿en qué medida el problema de su brazo, hombro o mano interfirió en su actividades sociales con la familia, amigos, vecinos o grupos? (Marque el número con un círculo)	Nada 1	Ligeramente 2	Moderadamente 3	Mucho 4	Extremadamente 5
23. Durante la semana pasada, ¿el problema de su brazo, hombro o mano limitó sus actividades laborales u otras actividades de la vida diaria? (Marque el número con un círculo)	Nada limitado 1	Ligeramente limitado 2	Moderadamente limitado 3	Muy limitado 4	Incapaz 5
Valore la gravedad de los siguientes síntomas durante la semana pasada (marque el número con un círculo)	Nula	Leve	Moderada	Severa	Extrema
24. Dolor en el brazo, hombro o mano	1	2	3	4	5
25. Dolor en el brazo, hombro o mano cuando realiza una actividad concreta	1	2	3	4	5
26. Sensación punzante u hormigueo en el brazo, hombro o mano	1	2	3	4	5
27. Debilidad en el brazo, hombro o mano	1	2	3	4	5
28. Rigidez en el brazo, hombro o mano	1	2	3	4	5
29. Durante la semana pasada, ¿cuánta dificultad tuvo para dormir a causa del dolor en el brazo, hombro o mano? (Marque el número con un círculo)	Ninguna dificultad 1	Dificultad leve 2	Dificultad moderada 3	Dificultad severa 4	Tanta dificultad que no pude dormir 5
30. Me siento menos capaz, con menos confianza y menos útil a causa del problema en el brazo, hombro o mano (marque el número con un círculo)	Totalmente en desacuerdo 1	En desacuerdo 2	Ni de acuerdo ni en desacuerdo 3	De acuerdo 4	Totalmente de acuerdo 5

Módulo de Deportes y Artes Plásticas (DASHe). Opcional

Las siguientes preguntas se refieren al impacto que tiene su problema del brazo, hombro o mano cuando toca un instrumento musical o practica deporte o en ambos casos. Si practica más de un deporte o toca más de un instrumento (o si practica un deporte y toca un instrumento), responda en relación con aquella actividad que sea más importante para usted. Si no practica deportes ni toca instrumentos musicales, no es necesario que rellene esta sección

Indique el deporte o el instrumento que sea más importante para usted:

Marque con un círculo el número que mejor describa su capacidad física durante la semana pasada. ¿Tuvo alguna dificultad...	Ninguna dificultad	Dificultad leve	Dificultad moderada	Dificultad severa	Incapaz
1.... para usar su técnica habitual al tocar el instrumento o practicar el deporte?	1	2	3	4	5
2.... para tocar el instrumento musical o para practicar el deporte a causa del dolor en el brazo, hombro o mano?	1	2	3	4	5
3.... para tocar el instrumento musical o para practicar el deporte tan bien como quisiera?	1	2	3	4	5
4.... para tocar el instrumento o practicar el deporte durante el tiempo que suele dedicar habitualmente a hacerlo?	1	2	3	4	5

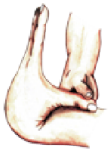
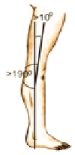
Módulo Laboral (DASHe). Opcional

Las siguientes preguntas se refieren al impacto que tiene su problema del brazo, hombro o mano sobre su capacidad para trabajar (incluido el trabajo doméstico, si es su tarea principal). Si no trabaja no es necesario que rellene esta sección

Indique en qué consiste su oficio/trabajo:

Marque con un círculo el número que mejor describa su capacidad física durante la semana pasada. ¿Tuvo alguna dificultad...	Ninguna dificultad	Dificultad leve	Dificultad moderada	Dificultad severa	Incapaz
1.... para usar su forma habitual de realizar su trabajo?	1	2	3	4	5
2.... para realizar su trabajo habitual a causa del dolor en el brazo, hombro o mano?	1	2	3	4	5
3.... para realizar su trabajo tan bien como quisiera?	1	2	3	4	5
4.... para realizar su trabajo durante el tiempo que suele dedicar habitualmente a hacerlo?	1	2	3	4	5

Appendix 7: Beighton test

Articulación		Hallazgo	Puntos
Quinto dedo izquiero		Dorsiflexión plasiva › 90º	1
		Dorsiflexión pasiva <= 90°	0
Quinto dedo derecho		Dorsiflexión plasiva › 90º	1
		Dorsiflexión pasiva <= 90°	0
Pulgar izquierdo		Toca el antebrazo con el pulgar	1
		No se aproxima hacia el antebrazo	0
Pulgar derecho		Toca el antebrazo con el pulgar	1
		No se aproxima hacia el antebrazo	0
Codo izquierdo		Hiperextiende › 10°	1
		Extensión <= 10	0
Codo derecho		Hiperextiende › 10°	1
		Extensión <= 10	0
Rodilla izquierda		Hiperextiende › 10°	1
		Extensión <= 10	0
Rodilla derecha		Hiperextiende › 10°	1
		Extensión <= 10	0
Flexión de tronco con rodillas extendidas		Palmas y manos llegan al suelo	1
		Palmas y manos no tocan el suelo	0

Total (sobre 9): Positivo si mayor que 6