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ORIGINAL ARTICLE

Human *COL5A1* rs12722 gene polymorphism and tendon properties in vivo in an asymptomatic population

Brandon Paul Foster · Christopher I. Morse · Gladys L. Onambele · Alun G. Williams

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Abstract
Purpose Gene variants encoding for proteins involved in homeostatic processes within tendons may influence its material and mechanical properties in humans. The purpose of this study was to examine the association between one such gene variant, gene encoding collagen type V alpha 1 chain (*COL5A1*) rs12722, and patellar tendon dimensions and mechanical properties in vivo.
Methods Eighty-four recreationally active, Caucasian, men and women, aged 18–39, with no history of injuries to the knee and a body mass index between 18.5 and 30 were recruited. Women were not recruited if they were pregnant or using any form of hormone-based contraception. The *COL5A1* rs12722 genotype was determined using real-time polymerase chain reaction. Patellar tendon dimensions (volume) and functional (elastic modulus) properties were assessed in vivo using geometric modelling, isokinetic dynamometry, electromyography and ultrasonography.
Results After adjustments for non-genetic factors, no significant associations were evident between the *COL5A1* rs12722 gene variant and either patellar tendon volume ($P = 0.933$) or elastic modulus ($P = 0.206$), nor with a calculated Z score that combined these dimensional and functional properties into a composite value ($P = 0.647$). Similarly, no association was evident when comparing individuals with/without the rare C allele (volume, $P = 0.883$; elastic modulus, $P = 0.129$; Z score, $P = 0.631$).
Conclusions Tendon properties do not seem to be influenced by the *COL5A1* rs12722 gene variant. Although the *COL5A1* rs12722 polymorphism has previously been associated with the risk of tendon pathology, that association is unlikely to be mediated via underlying tendon dimensional and functional properties.

Keywords Genetic association studies · Gene variants · Tendon properties · Asymptomatic · In vivo

Abbreviations

AT	Achilles tendinopathies
BF	Biceps femoris
BMI	Body mass index
CcT	Co-contraction torque
Col V	Collagen type V
COL5A1	Gene encoding collagen type V alpha 1 chain
CSA	Cross-sectional area
DNA	Deoxyribonucleic acid
DEXA	Dual X-ray absorptiometry
E	Elastic modulus
EDTA	Ethylene diamino tetra-acetic acid
ELISA	Enzyme-linked immunosorbent assay
EMG	Electromyography
E2	Oestradiol
F_{Max}	Maximal force
GPa	Gigapascals
HWE	Hardy–Weinberg equilibrium
K	Stiffness
mm^3	Cubic millimetres
MRI	Magnetic resonance imaging
MVC	Maximal voluntary contraction

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B. P. Foster (✉)
Maternal and Fetal Health Research Group, Institute of Human Development, St. Mary's Hospital, University of Manchester, 5th Floor, Oxford Road, Manchester M13 9WL, UK
e-mail: brandon.foster-2@manchester.ac.uk

C. I. Morse · G. L. Onambele · A. G. Williams
Institute for Performance Research, Manchester Metropolitan University, Crewe CW1 5DU, UK

PCR	Polymerase chain reaction
pg	Picogram
PTL	Patellar tendon length
PTMA	Patellar tendon moment arm
ROM	Range of motion

Introduction

The interaction between muscle and tendon influences not only force transmission (Burgess et al. 2007; Reeves et al. 2003), but also energy storage and return for locomotion (Alexander 1991; Voigt et al. 1995; Fukunaga et al. 2001), joint positional control (Loram et al. 2004, 2005a, b), and to protect from muscle fibre damage (Griffiths 1991; Lieber and Friden 2000). Therefore, the tendon mechanical properties play a pivotal role in determining the function of the overall muscle–tendon complex. The tendon mechanical property most commonly associated with in vivo function is the 'elastic modulus (*E*)', i.e. the relation between stress and strain. *E* represents the material properties of tendon independent of its dimensions, making it possible to compare tendon mechanical properties between individuals with different tendon dimensions. Essentially, a high tendon *E* represents a relatively stiff tissue.

Recently, it has been reported that a gene variant within the 3′-UTR of the *COL5A1* gene is associated with tendon pathologies (Mokone et al. 2006; September et al. 2008), range of motion (ROM) (Collins et al. 2009; Brown et al. 2011b), endurance running performance (Posthumus et al. 2011) and tendon mechanical properties of the knee extensors (Kubo et al. 2013). The *COL5A1* gene encodes the pro α1 chain of type V collagen (Col V), a quantitatively minor fibrillar collagen that through its heterotypic interactions with type I collagen (Col I), may have regulatory roles in controlling fibril diameter within connective tissues such as tendon (Birk et al. 1990; Wenstrup et al. 2011). In particular, the CC genotype of the *COL5A1* rs12722 gene variant was over-represented in asymptomatic participants compared with chronic Achilles tendinopathies (AT) in two independent Caucasian populations [South Africa (Mokone et al. 2006) and Australia (September et al. 2008)]. Similarly, the CC genotype was associated with increased sit and reach ROM (Brown et al. 2011a, c), although Collins et al. (2009) reported that the CT genotype had lower sit and reach, and standing leg raise ROM, than individuals homozygous for either allele. The TT genotype of the *COL5A1* rs12722 gene variant has been associated with enhanced endurance running performance (Posthumus et al. 2011; Brown et al. 2011a). In addition, the CC genotype has been associated with more extensible tendon structures of the knee extensors in a population of Japanese males (Kubo et al. 2013).

A biological link has recently been proposed between the rs12722 gene variant within the 3′-UTR of *COL5A1* and the gene product, Col V (Laguette et al. 2011). Specifically, it has been reported that the function of the TT and CC genotypes is to increase and decrease the stiffness of the tissue, respectively, through changes in *COL5A1* mRNA stability, within normal physiological ranges (Collins and Posthumus 2011). It may also be that increased *COL5A1* mRNA stability related to the TT genotype of the *COL5A1* rs12772 gene variant results in increased levels of Col V and reduced fibril diameter, as reported in in vitro assays (Birk et al. 1990). However, just how this directly or indirectly affects the global dimensions and volume, as well as the mechanical properties of tendon remains to be elucidated.

The multifactorial nature of tendon pathologies (Riley 2004), ROM (Gleim and McHugh 1997) and endurance running performance (Joyner and Coyle 2008), makes it difficult to identify the main causative factors that contribute to the phenotype, although tendon stiffness may be one such intermediate phenotype linking genetic variation to risk of injury, ROM and endurance running performance. Independent of genetics, relationships appear to exist between these phenotypes for example, ROM has been associated with tendon injuries (Witvrouw et al. 2004, 2007), a more compliant tendon (low stiffness) might be able to absorb more energy for a given mechanical load, thus reducing the risk of strain overload (Witvrouw et al. 2004), and an inverse relationship between running economy and tendon stiffness has been reported (Arampatzis et al. 2006; Fletcher et al. 2010). The *COL5A1* rs12722 gene variant might be associated with morphological changes to the collagen fibrils that directly or indirectly modify the tendon mechanical properties.

The mechanical properties of tendon can now be assessed in vivo in humans with high accuracy and reliability, as exemplified by Pearson and Onambele (2006). This kind of direct in vivo assessment of tendon properties contrasts with the previous surrogate measures of tendon properties obtained from sit and reach and standing leg raise tests when investigating the *COL5A1* rs12722 gene variant (Collins et al. 2009; Brown et al. 2011b). Sit and reach and standing leg raise tests are commonly used tests of musculoskeletal function but are not measurements of tendon properties per se.

This study therefore seeks to advance our understanding of the genetic components associated with physical performance parameters and risk of musculoskeletal injuries, by assessing tendon mechanical properties. Thus, the aim of this study was to investigate for the first time whether the *COL5A1* rs12722 gene variant is associated with the *E* of the patellar tendon, in an asymptomatic Caucasian population, using an accurate, reproducible and non-invasive

assessment of tendon properties in vivo. Additional aims were to investigate whether the dimensions of the tendon, and the composite effect of both *E* and volume, are associated with this gene variant.

Method

Participants

Eighty-four healthy and recreationally active (at least 1.5 h/week) Caucasians were recruited (45 men and 39 women), aged [mean (SD)] 23 (4) years, height 173 (10) cm, body mass 71.6 (12.4) kg, and BMI 24 (2.8) kg/m^2.

To minimise confounding factors, potential participants were not recruited if they: were non-Caucasian; were very sedentary or very active (completed less than 2.5 h/week or more than 5 h/week of moderate exercise (Tucker et al. 2011) as determined by a questionnaire); had any current or recent lower limb injuries in the past year before testing, including tendinopathies of the patellar tendon; were aged under 18 or over 40 years; were diabetic; were smoked regularly; were regular users of medication; or had a body mass index (BMI) outside the range 18.5–30 kg/m^2. Female volunteers were not recruited if pregnant or using hormone-based contraception. The investigation was approved by the local ethics committee at Manchester Metropolitan University, and all participants gave their written informed consent to participate. The study conforms to the latest revision of the Declaration of Helsinki.

Measurement of tendon dimensional and mechanical properties

Maximal patellar tendon isometric force

All measurements of torque were carried out on an isokinetic dynamometer (Cybex, Phoenix Healthcare, UK) on the left leg. The knee was fixed at 90° flexion (full extension = 0°) and hip angle at 85° (supine position = 0°). The position and strapping were, as per the manufacturer's guidelines, designed to minimise any extraneous movements. Participants were instructed to perform ramp isometric knee extensions to maximum over a 5–7 s period. Maximal tendon force was calculated as described previously (Eq. 1), (Pearson and Onambele 2006; Onambele-Pearson and Pearson 2007)

$$F_{\mathrm{Max}} = (\mathrm{MVC_{KE}} + \mathrm{CcT}) / \mathrm{PTMA} \quad (1)$$

where F_{Max} is the maximal patella tendon force, $\mathrm{MVC_{KE}}$ the maximal isometric knee-extensor torque (i.e. the measured torque during testing), CcT the knee flexion torque of the hamstrings during knee extension (antagonist co-contraction torque—see next section for calculation), and PTMA the patellar tendon moment arm (see sections below).

Estimation of antagonist co-contraction using electromyography

Electromyographic (EMG) activity was assessed using a pair of self-adhesive Ag–AgCl electrodes (Ambu Neuroline 72000-S/25, Ballerup, Denmark) placed in a bi-polar configuration (Zipp 1982), at a site corresponding to the distal one-third on the long head of the biceps femoris (BF) (representative muscle of the knee flexors). The raw EMG signal was collected at a frequency of 2,000 Hz, pre-amplified (2,000×), band-pass filtered between 500 and 10 Hz by the same system that processed the torque data (Acknowledge, Biopac Systems, Santa Barbara, CA, USA) and displayed in real-time on the same output graph (iMac, Apple, California). The root mean square (RMS) EMG activity corresponding to the peak torque period was analysed and averaged for a 500-ms period during the plateau of peak torque (i.e. 250 ms either side of the instantaneous peak torque). These EMG data were used as a measure of antagonist co-contraction (i.e. CcT) during isometric knee extensions (Reeves et al. 2003), calculated as the product of BF EMG activity during $\mathrm{MVC_{KE}}$, divided by BF peak flexor EMG at 90° knee flexion MVC and multiplied by hamstrings maximal flexion torque.

Patellar tendon displacement

Patellar tendon displacement was determined using real-time B-mode ultrasonography (AU5, Esaote, Biomedica, Italy) as described previously (Onambele et al. 2007). Briefly, during a ramp isometric knee extension performed over 5–7 s with the knee fixed at 90° flexion, the ultrasound probe (7.5 MHz linear array probe, 40 mm wide) was positioned in a sagittal plane over the patellar tendon alternatively over; (a) patella proximal (inferior pole of the patella) or (b) tibia distal (tibial tuberosity) excursions, so that the sum of tibial and patellar displacements would be computed as total displacement. Total displacements were determined at 10 % force level intervals (from 10 to 100 %) using digitising software (Kinovea, version 0.8.15, Joan Charmant & Contributors, France), consistent with others (Onambele et al. 2007). This method is widely used and has high reliability [e.g. (Pearson and Onambele 2006; Kubo et al. 2001; Reeves et al. 2003)]. Measurements were taken after five preconditioning contractions to ensure reproducibility (Maganaris 2003) and early afternoon, to minimise variability in tendon stiffness related to time of day (Pearson and Onambele 2006).

Patellar tendon moment arm length

Patellar tendon moment arm length (PTMA) was measured from an 11 s sagittal plane scan of the left leg of each participant at rest, using a single, low-energy X-ray beam (0.9 μSv) protocol on a DEXA (Dual X-ray Absorptiometry) scan (Hologic QDR, Vertec, Reading, UK). For the imaging limb, the participant lay on their side with the left hip and knee flexed at 90° so that the source detector probes could pass across the knee within a 20 cm scanning window. The PTMA was defined as the perpendicular distance from the patellar tendon to the mid-point of the distance between the estimated tibiofemoral contact points in the lateral and medial femoral condyles (Baltzopoulos 1995; Tsaopoulos et al. 2006). The previously validated MRI for measuring PTMA indicates a very strong relationship with DEXA ($n = 10$, $r^2 = 0.962$, $P = 0.001$, unpublished data).

Calculation of patellar tendon stiffness

Patellar tendon stiffness (K, N mm^{-1}) was calculated from the slope of the tangents of the force–displacement relations (at 10 % force intervals), which were fitted with a second-order polynomial function forced through zero. The 10 % force intervals derive from the estimated maximum force (F_{Max}) experienced by the tendon during the ramp MVC (see Eq. 1). The displacement of the tendon was measured as described previously. In addition, to allow for stiffness comparisons at an absolute load across populations, tendon stiffness was also calculated at a standardised force level which corresponded to just under the maximum baseline value of the weakest person (male = 1,067 N; female = 1,034 N).

Calculation of elastic modulus

Patellar tendon cross-sectional area (PTCSA) and patellar tendon length (PTL) were measured in the resting state at a knee joint angle of 90°. PTCSA was determined from the mean of transverse-plane ultrasound images taken at 25, 50 and 75 % of patellar tendon length, and processed using digitising software (Image J, National Institute of Health, Bethesda, MD, USA). PTL was assessed from sagittal-plane ultrasound images and measured from the inferior pole of the patella to the superior aspect of the tibial tuberosity. Elastic modulus (E) in GPa was calculated by multiplying K by the ratio of PTL to PTCSA ($E = K \times (\text{PTL} \div \text{PTCSA})$).

Calculation of tendon volume

Patellar tendon volume (PTV) was calculated by geometric principles assuming a uniformly tapering truncated cone between measurement positions (i.e. the product of PTCSA at the three sections of the tendon, 25, 50, 75 %, and PTL). Muscle and tendon geometry have previously been modelled using similar methods (Jones and Pearson 1969; Fuller et al. 1999; Tothill and Stewart 2002).

***Z** score analyses*

To provide a stable measure of the overall association of genotype with tendon properties, composites were formed with unit-weighted Z scores of constituent tests (Ackerman and Cianciolo 2000), i.e. elastic modulus and tendon volume. Hence, the dimensional (volume) and functional (elastic modulus) properties of tendon could be scaled and analysed simultaneously. Thus, the raw test scores of E (GPa) and volume (mm^3) were converted to Z scores using Eq. 2:

$$\begin{aligned} Z\text{-score} &= (\text{variable score} - \text{mean})/\text{standard deviation} \\ Z\text{-score}_{\text{Composite}} &= Z\text{-score}_E + Z\text{-score}_{\text{PTV}} \end{aligned} \quad (2)$$

Genetic analysis

Sample collection

Buccal cells were collected using mouth swabs (Whatman Sterile OmniSwab, GE Healthcare, USA). Samples were immediately stored at −20 °C until DNA extraction.

DNA extraction

Standard procedures for genomic DNA isolation from buccal swabs were carried out using the Qiagen QIAcube spin column protocol and buffers in the Qiagen DNA Blood Mini kit (Qiagen, West Sussex, UK). Eluted DNA concentrations were ~10–30 ng/μL.

DNA quantification

The concentration and purity of the sample was calculated using a biophotometer (WPA UV1101, Biochrom, Cambridge, UK). Briefly, ~12 μL of the DNA sample was pipetted into a glass cuvette, the absorbance readings of ultraviolet light at wavelengths of 260 and 280 nm were performed, and the 260/280 nm ratio was determined. Good quality DNA will have a ratio of 1.7–2.0 (Glasel 1995), and all samples fell within these ratios.

COL5A1 genotyping

COL5A1 rs12722 genotyping was carried out in the genetics laboratory of the Exercise and Sports Science complex as part of Crewe Campus, Manchester Metropolitan

University, England. Genotypes were determined using fluorescence-based TaqMan real-time polymerase chain reaction (PCR). Predesigned primers and allele-specific probes specific to the 'C' allele (VIC) and 'T' allele (FAM) were used (Applied Biosystems, Foster City, CA, USA). The assay volume within each well of a 96-well PCR plate (Bio-Rad Laboratories Ltd, Herts, UK) was 10 μL, which included 1 μL of purified DNA, 5 μL of 2× TaqMan genotyping master mix (Applied Biosystems), 0.5 μL of 20× genotyping assay (Applied Biosystems) and 3.5 μL nuclease-free H_2O (Qiagen). The PCR plate was sealed using MicroSeal 'B' Adhesive Seals (Bio-Rad) and ran on a Chromo4 Real-Time PCR Detection System (Bio-Rad) for 10 min at 95 °C. This was followed by 40 cycles of denaturing at 92 °C for 15 s, primer annealing and extension at 60 °C for 60 s, and plate read. Genotypes were determined by endpoint fluorescence of VIC and FAM signals using the Chromo4 PCR machine, and results were analysed using Opticon Monitor Software version 3.1.32 (Bio-Rad). All analyses were run in duplicate, there was 100 % agreement between duplicate wells, and genotyping was completed in all samples.

Hardy–Weinberg equilibrium

Genotype data were tested for Hardy–Weinberg equilibrium (HWE), using a freely available software package (Rodriguez et al. 2009). This test was conducted on the initial cohort before selecting individuals for the phenotype tests (based on a higher degree of homozygosity), in order to establish whether the genotype and allele frequencies were constant between the initial cohort and the general population. Indeed, the genotype data for this cohort were in HWE [$P > 0.05$ with 1 *df* (one degree of freedom)].

Oestradiol measures

It has been reported that oestradiol levels are associated with tendon mechanical properties in vivo (Burgess et al. 2009). Therefore, following the measures of tendon properties, female participants only reported to the biochemistry laboratory where whole blood (5 mL) was drawn from a superficial forearm vein into serum separator tubes containing anti-coagulant (EDTA) (Sarstedt Monovette-Red cap, Numbrecht, Germany). After storage on ice for ~30 min, the blood was centrifuged at 2–5 °C for 10 min at 4,100 rpm, with the supernatant extracted (~2 mL) and stored at −20 °C for later analysis. Serum 17ß-oestradiol (E2) was quantitatively determined using standard enzyme-linked immunosorbent assay (ELISA) procedures (Alpha Diagnostic International, San Antonio, USA; minimal detectable conc. of ~10 pg/mL, intra-assay precision of 9.9 %, inter-assay precision of 10.1 %). E2 concentration at day 1 of the menstrual cycle was extrapolated using data from women with similar characteristics to those in the current study (i.e. age range 20–36 years, no use of contraceptives) (Stricker et al. 2006). Extrapolated serum oestradiol in the 39 female participants was therefore 34.0 (30.0) pg/mL.

Statistical power to detect genotype–phenotype associations

Once the participant subgroup had been identified, it was prudent to perform a priori, statistical procedures to estimate the extent to which trait variation (i.e. tendon properties) is explained by the *COL5A1* rs12722 gene variant. So, based on power calculations with alpha set at 0.05 and beta set at 0.80 and using mean and standard deviation data of tendon properties obtained in our laboratory, it was estimated that approximately 80 of the original 160 participants would be required to complete the tests of tendon properties, in order to detect differences in tendon properties in the order of ~1–2 % for tendon volume, and ~10–15 % for tendon modulus between the three genotype groups. G*Power 3.1.6 (Franz Faul, Universitat Kiel, Germany) was used to calculate sample size.

Statistical analyses

Reliability was evaluated using ratio limits of agreement (Nevill and Atkinson 1997) to quantify the absolute reliability or 'agreement' between measurements on separate occasions. All data were analysed with SPSS version 19.0.0. One-way analysis of variance (ANOVA) was performed on all three genotype groups and the measures of PTV and *Z* scores. In addition, independent *t* tests were performed on volume and *Z* scores when combining heterozygotes with the smallest homozygote group. The Kruskal–Wallis non-parametric equivalent statistical test was performed on *E* and its association with the three genotype groups, while the Mann–Whitney *U* test was used to compare *E* between one homozygote group and the other combined genotype group. PTV differed between sexes, and BMI was correlated with both PTV and *Z* score, so were used as covariates accordingly. Age and oestradiol concentration showed no correlation with any phenotype so were not used as covariates. Alpha was set at 0.05. Unless otherwise stated, data are presented as mean (standard deviation).

Results

There were no significant differences in age, height, mass and BMI between the three genotype groups, as well as between the TT genotype group and TC and CC combined genotype groups (Table 1).

Table 1 Age and physical characteristics of all participants according to genotypes of the *COL5A1* rs12722 gene variant

	All	TT	TC	CC	*P* value	TC + CC	*P* value
n	84	26	45	13	–	58	–
Age (years)	23.1 (4.0)	21.8 (1.8)	23.4 (5.2)	22.9 (3.1)	0.360	23.1 (4.1)	0.175
Sex (% male)	53.6	46.2	62.2	38.5	0.991	56.9	0.367
Height (cm)	173.0 (10.0)	171.8 (7.6)	171.9 (6.7)	173.0 (8.7)	0.756	172.5 (7.7)	0.427
Mass (kg)	71.6 (12.8)	70.3 (8.9)	71.1 (10.6)	71.2 (10.0)	0.706	71.15 (10.3)	0.368
BMI (kg m^{-2})	24.0 (2.8)	24.3 (2.9)	24.1 (2.5)	22.9 (2.6)	0.127	23.5 (2.55)	0.527

The *P* values of the TT versus TC versus CC, and TT versus TC/CC combined genotype groups derive from a one-way ANOVA, and independent *t* test, respectively

Table 2 Patellar tendon properties for the *COL5A1* rs12722 genotype groups

$n = 84$	*COL5A1* rs12722 genotype			
	TT ($n = 26$)	TC ($n = 45$)	CC ($n = 13$)	*P* value
CSA (mm^2)	74.9 (15.2)	78.2 (13.1)	70.8 (15.5)	0.200
Tendon length (mm)	49.6 (4.6)	50.8 (4.3)	49.5 (5.0)	0.587
Maximal displacement (mm)	13.0 (4.0)	13.5 (4.1)	13.3 (5.9)	0.915
Maximal strain (%)	6.5 (2.2)	6.9 (2.3)	6.5 (2.5)	0.803
Maximal stiffness (N mm^{-1})	855.7 (1,569.1)	707.6 (1,729.1)	555.3 (1,261.5)	0.203
Comparison stiffness (N mm^{-1})	531.9 (732.9)	457.9 (1,005.9)	441.1 (776.7)	0.394
Volume (mm^3)	1,879 (522)	1,999 (424)	1,773 (522)	0.933
Elastic modulus (GPa)	0.54 (1.12)	0.44 (1.23)	0.37 (0.39)	0.206
Z score	0.17 (1.67)	0.08 (1.29)	−0.57 (1.71)	0.647

Maximal stiffness, comparison stiffness and elastic modulus are expressed as median (range)

Between-day measurement reliability was examined using ratio limits of agreement. PTV showed no bias and excellent agreement (×/÷ 1.019). *E* showed no bias and very good agreement (×/÷ 1.144).

As oestradiol showed no significant correlation with tendon properties (CSA, $r = 0.155$, $P = 0.345$; tendon length, $r = -0.243$, $P = 0.137$; maximal displacement, $r = -0.258$, $P = 0.113$; maximal strain, $r = -0.171$, $P = 0.298$; maximal stiffness, $r = 0.148$, $P = 0.368$; comparison stiffness, $r = 0.267$, $P = 0.100$; volume, $r = 0.010$, $P = 0.950$; *E*, $r = 0.146$, $P = 0.375$; *Z* scores, $r = 0.055$, $P = 0.740$) and having adjusted for non-genetic factors such as sex and BMI, male and female participants data were therefore pooled into a single population. There were no significant differences in patellar CSA, tendon length, maximal displacement, maximal strain, and stiffness at a common force level, or indeed, tendon volume and *E* or *Z* scores, between the genotype groups (CSA, $P = 0.200$; tendon length, $P = 0.587$; maximal displacement, $P = 0.915$; maximal strain, $P = 0.803$; maximal stiffness, $P = 0.203$; comparison stiffness, $P = 0.394$; volume, $P = 0.933$; *E*, $P = 0.206$; *Z* scores, $P = 0.820$) (Table 2). In addition, there were no significant differences in CSA, tendon length, maximal displacement, maximal strain and stiffness (CSA, $P = 0.636$; tendon length, $P = 0.384$; maximal displacement, $P = 0.768$; maximal strain, $P = 0.640$; maximal stiffness, $P = 0.262$; comparison stiffness, $P = 0.238$) or for measures of volume, *E* or *Z* scores (volume, $P = 0.883$; *E*, $P = 0.129$; *Z* scores, $P = 0.631$) (Fig. 1), when comparing the TT genotype group to the combined TC and CC genotype groups. The TC and CC genotype groups were combined due to the small CC group size.

Discussion

This study reports no association between the *COL5A1* rs12722 gene variant and tendon properties. The *COL5A1* rs12722 variant does not associate with dimensional (volume) and functional (*E*) properties of the patellar tendon, or indeed as a composite (*Z* score), in an asymptomatic Caucasian population. In this study, patellar tendon modulus was comparable with other data for this phenotype in healthy, young, male and female subpopulations, ~0.5 GPa compared with ~0.5–0.6 GPa (O'Brien et al. 2010) and ~0.75 GPa (Hsin-Yi and Paul 2010). The larger sample size used in the present study ($n = 84$) compared with those aforementioned ($n = 10$–20) gives us increasing confidence that the modulus data are representative of the wider population. No previous study has reported PTV.

An association between the *COL5A1* rs12722 variant and patellar tendon properties was hypothesised on the role

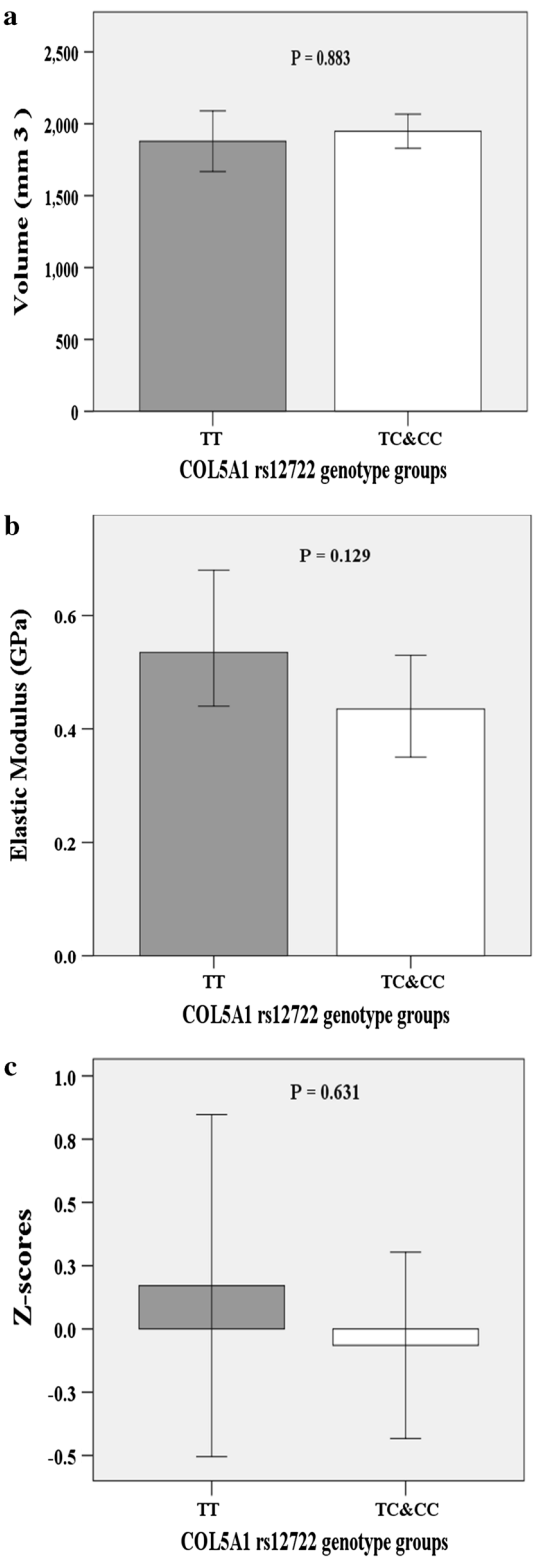

Fig. 1 Patellar tendon properties for *COL5A1* rs12722 genotype groups. **a** Volume. **b** Elastic modulus. **c** *Z* scores. Volume and *Z* scores are expressed as mean (SD). Elastic modulus is expressed as median (range)

of *COL5A1*. The *COL5A1* gene encodes the pro α1 chain of type V collagen (Col V) which, through its heterotypic interactions with type I collagen (Col I), may have regulatory roles in controlling fibril diameter within connective tissues such as tendon (Birk et al. 1990; Wenstrup et al. 2011). Specifically, it may be that increased *COL5A1* mRNA stability related to the TT genotype of the *COL5A1* rs12722 gene variant results in increased levels of Col V, reduced fibril diameter and increased stiffness of the tissue, within normal physiological ranges (Collins and Posthumus 2011). However, in this study, no differences were evident in dimensional and functional properties between the genotype groups (Table 2).

Previous reports have associated the *COL5A1* rs12722 variant with phenotypes such as Achilles tendon pathologies (Mokone et al. 2006; September et al. 2008), ROM (Brown et al. 2011b; Collins et al. 2009) and endurance running performance (Brown et al. 2011a; Posthumus et al. 2011); phenotypes that could be influenced by tendon *E*. Indeed more recently, tendon mechanical properties related to higher maximal displacements and strain rates, and lower stiffness have been associated with the CC genotype of the *COL5A1* rs12722 gene variant in a Japanese, male population (Kubo et al. 2013). However, we observed no link between the *COL5A1* gene variant and these tendon mechanical properties, as well as *E*. These differences in observations between the previous study (Kubo et al. 2013) and our own may be due to a number of methodological disparities. Firstly, they measured from the aponeurosis and corrected to calculate tendon elongation, so it is possible where we have seen no association with patellar tendon properties and the *COL5A1* gene variant, it is due to an influence on the collagenous material proximal and in series to the tendon. Secondly, their stiffness calculations do not account for both the proximal and distal displacements of the tendon and so may underestimate the total displacement and hence stiffness of the tendon under loading (Hansen et al. 2006; Onambele et al. 2007). Thirdly, *E* was not calculated in their study, although variability in their stress and strain values may have been associated with measurements at MVC, as opposed to standardised force values in our calculations. However, our data are consistent with theirs on tendon mechanical properties of the plantar flexors, in that there were no associations with the *COL5A1* gene variant. There is also likely to be ethnic differences in genotype–phenotype interactions between the Asian cohort and the Northern European cohort used in this study. Indeed, muscle stiffness of the triceps surae can differ significantly between ethnic groups; thus, direct comparisons of tendon mechanical properties between these groups might also be problematic (Fukashiro et al. 2002).

Collins and Posthumus (2011) proposed an association between injury, ROM and endurance running ability phenotypes, and the *COL5A1* rs12722 variant, with mechanical properties of musculoskeletal soft tissue being a possible intermediate phenotype. In this investigation, we assessed tendon *E* under isometric conditions, and although it is an established method for determining material properties in vivo precisely, it may be limited in describing implications for the muscle–tendon complex as a whole. For example, although endurance running performance has been associated with the *COL5A1* rs12722 gene variant and here we report no association with tendon *E* or geometry, it is perhaps relevant to note that tendon hysteresis (viscoelastic property relating to energy economy) as opposed to *E* is likely to influence endurance running performance (Sano et al. 2012). Indeed, with regards to injury, there remains no clear link between tendon properties and tendon or muscle damage. There are no data describing the relationship between patellar tendon *E* per se and either predisposition to injury or flexibility/ROM. In addition, the patellar tendon may play a different functional role than the Achilles tendon—the Achilles contributes significantly to ROM (Morse et al. 2008), and due to its greater length, contributes significantly to attenuating muscle length changes during eccentric loading (Spanjaard et al. 2008).

Phenotypes such as ROM, running performance and risk of injury are certainly multifactorial, which includes both genetic and several non-genetic factors. This makes associating inter-individual genetic variation with phenotypes such as tendon properties more difficult. Compounding this complexity is the fact that the relevant non-genetic factors can be considered multifactorial phenotypes in their own right (Collins and Raleigh 2009). In this study, an attempt was made to maximise the ability to detect genotype–phenotype associations by controlling for non-genetic factors and variables known to contribute to the variability on tendon dimensions and function, by adopting strict exclusion criteria. For instance, an asymptomatic group of participants with a limited range of age, BMI, geographic ancestry and no history of lower limb injuries were studied, while other potentially confounding factors such as variation in circulating concentrations of female reproductive hormones (due to natural variation or exogenous sources) were also controlled during recruitment and data analysis. Despite these efforts, we observed no genotype–phenotype association.

Potential limitations of this study were the relatively small sample size ($n = 84$) for a genotype–phenotype association study. However, power calculations performed a priori showed the sample size was sufficient to detect differences in tendon properties between three genotypes, in the order of <1 % for volume and ~7–8 % for *E*. Nevertheless, a larger sample size is encouraged in future research to increase the statistical power yet further, and thus the ability to detect even more subtle genotype–phenotype associations, particularly when investigating the contribution of a single genetic marker. Secondly, tendon CSA measures were taken at 25, 50 and 75 % of the tendon length and not 0 or 100 %, so it is possible that the volume was underestimated which would have affected subsequent calculations of *E*. While the most likely scenario is that our observation of no genotype–phenotype association is a true negative, given that this study is the first direct investigation of a potential association between the *COL5A1* rs12722 gene variant and patellar tendon properties, it remains a possibility that weak associations do exist. Even if that does prove to be the case, the functional significance of any future statistically significant observation is likely to be very low.

In conclusion, there was no association between the *COL5A1* rs12722 gene variant and measures of patellar tendon properties in an asymptomatic cohort. Nevertheless, DNA sequence variants within genes with structural and regulatory roles in the tendon extracellular matrix, including variants not yet attracting interest in this context, should continue to undergo investigation for their potential influences on tendon properties. Tendons of the lower limbs including the patellar and Achilles tendon remain of most interest because of their central role in ambulation across the spectrum of health and disease. More powerful multifactorial models which include molecular factors that contribute to physical performance and tendon pathologies would be extremely useful.

Conflict of interest None.

Ethical standard Ethics approval was provided by Human Research Ethics Committee of Manchester Metropolitan University.

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