

Polymorphisms in *PTK2* are associated with skeletal muscle specific force: an independent replication study

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Abstract

Purpose The aim of the study was to investigate two single nucleotide polymorphisms (SNP) in *PTK2* for associations with human muscle strength phenotypes in healthy men.

Methods Measurement of maximal isometric voluntary knee extension (MVC_{KE}) torque, net MVC_{KE} torque and vastus lateralis (VL) specific force, using established techniques, was completed on 120 Caucasian men (age = 20.6 ± 2.3 year; height = 1.79 ± 0.06 m; mass = 75.0 ± 10.0 kg; mean \pm SD). All participants provided either a blood ($n=96$) or buccal cell sample, from which DNA was isolated and genotyped for the *PTK2* rs7843014 A/C and rs7460 A/T SNPs using real-time polymerase chain reaction.

Results Genotype frequencies for both SNPs were in Hardy–Weinberg equilibrium ($X^2 \leq 1.661$, $P \geq 0.436$). VL specific force was 8.3% higher in rs7843014 AA homozygotes than C-allele carriers ($P=0.017$) and 5.4% higher in rs7460 AA homozygotes than T-allele carriers ($P=0.029$). No associations between either SNP and net MVC_{KE} torque ($P \geq 0.094$) or peak MVC_{KE} torque ($P \geq 0.107$) were observed.

Conclusions These findings identify a genetic contribution to the inter-individual variability within muscle

specific force and provides the first independent replication, in a larger Caucasian cohort, of an association between these *PTK2* SNPs and muscle specific force, thus extending our understanding of the influence of genetic variation on the intrinsic strength of muscle.

Keywords Protein tyrosine kinase 2 · Vastus lateralis · Lateral transmission · Focal adhesion kinase · Muscle quality · Genetic polymorphism

Abbreviations

ACSA	Anatomical cross-sectional area
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
AVI	Audio video interleave
BMI	Body mass index
CcT	Co-activation torque
DXA	Dual energy X-ray absorptiometry
ECM	Extracellular matrix
EMG	Electromyography
FAK	Focal adhesion kinase
MVC_{KE}	Maximal voluntary isometric knee extension
MVC_{KF}	Maximal voluntary isometric knee flexion
PCSA	Physiological cross-sectional area
PTK2	Protein tyrosine kinase 2
SNP	Single nucleotide polymorphism
STT	Superimposed twitch torque

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Introduction

Muscle specific force, defined here as the force per unit cross-sectional area of muscle, represents the intrinsic strength of a muscle and is a key determinant of functional capacity and mobility in later life which should be

maintained (McGregor et al. 2014). In young, untrained adults muscle specific force varies inter-individually by 14–16% (Stebbins et al. 2014; Erskine et al. 2009), which might suggest some individuals are predisposed to possessing greater muscle specific force and may, therefore, be less susceptible to experiencing functional declines associated with ageing. Whilst it is generally accepted that differences in the force generating capacity of individual fibres (Bottinelli et al. 1996), intramuscular fat and/or connective tissue content (Macaluso et al. 2002; Kent-Braun et al. 2000) contribute to this inter-individual variability, it is possible that genetic differences also contribute. Accordingly, two single nucleotide polymorphisms (SNPs) of the protein tyrosine kinase 2 gene (*PTK2*), responsible for encoding focal adhesion kinase (FAK), were found to explain ~10% of the inter-individual variability in the muscle specific force of untrained individuals (Erskine et al. 2012).

Focal adhesion kinase is an integrin-associated protein tyrosine kinase localised at focal adhesion complexes via interactions between its focal adhesion targeting domain and other integrin-associated proteins (Hildebrand et al. 1993; Schaller 2001). Focal adhesion complexes are important components of cell costameres, which overlie the Z- and M-lines of myofibres and form regular connections with the sarcolemma (Pardo et al. 1983). Thus, a pathway of force transmission exists by which force can be transmitted laterally across adjacent myofibres and the sarcolemma to the extracellular matrix (ECM) (Flück et al. 1999; Patel and Lieber 1997; Bloch and Gonzalez-Serratos 2003), via these regular connections. Given the location of costameres, they could effectively split the serial sarcomeres of a myofibre into multiple force-generating units to enable this pathway of force transmission to the muscle fascia, although this hypothesis is speculative (Jones et al. 1989; Huijing 1999). Costamere formation and turnover is regulated by FAK activity, which is known to increase in hypertrophying muscle (Flück et al. 1999). Similarly, increases in muscle specific force are also observed following skeletal muscle hypertrophy (Reeves et al. 2004b), which is thought to be the consequence of an increased costamere density (Erskine et al. 2012). A greater costamere density, that is an increase in the number of intermediate connections between the ECM, myofibre and aponeurosis, may result in an increase in the number of force-generating units within a fibre, and thus, whole muscle, without the need for additional parallel sarcomeres. Consequently, the force generated by the muscle fibre and whole muscle would be greater despite physiological cross-sectional area (PSCA) remaining constant, thus enhancing muscle specific force. Indeed where alterations to normal costamere function have been observed in dystrophic muscle, decreased

specific force was attributed to an inferior ability to transmit force laterally due to deficiencies in important costameric proteins (Ramaswamy et al. 2011).

The *PTK2* rs7843014 and rs7460 polymorphisms, which are in linkage disequilibrium (Erskine et al. 2012), were recently associated with the level of gene expression and exceptional longevity in a Spanish population (Garatachea et al. 2014). More specifically, the rs7843014 CC and rs7460 TT genotypes were tentatively associated with lower *PTK2* gene expression whilst being optimal for exceptional longevity (Garatachea et al. 2014). In a population of healthy Caucasian men, however, it was the rs7843014 AA and rs7460 AA genotypes that were significantly associated with producing higher baseline specific force (Erskine et al. 2012). The authors speculated that the rs7843014 AA and rs7460 AA homozygotes had improved lateral force transmission due to an increased costamere density compared to C-allele and T-allele carriers, respectively (Erskine et al. 2012). This preliminary report, however, involved only a small population ($n=51$), therefore, independent replications are required to confirm these initial observations.

The aim of the current investigation, therefore, was to investigate if the rs7843014 and rs7460 polymorphisms within the *PTK2* gene were associated with skeletal muscle specific force and other measures of muscle strength in a larger population of Caucasian men, independent of that done previously. We hypothesized that the rs7843014 AA and rs7460 AA genotypes would be significantly associated with higher specific force, maximal isometric voluntary knee extension (MVC_{KE}) torque and net MVC_{KE} torque production than C-allele and T-allele carriers, respectively.

Methods

Participants

Participants were healthy, unrelated Caucasian men ($n=120$, Table 1) who had not undertaken any structured training in the preceding 12 months and had not taken part in the aforementioned preliminary report (Erskine et al. 2012). Participants were free from musculoskeletal or neurological disorders, had a body mass index (BMI) between 18.5 and 30.0 kg m^{-2} and all gave written consent to participate. Habitual physical activity levels were assessed via questionnaire (Baecke et al. 1982) to ensure only those participants who undertook less than 3 h of low-to-moderate habitual work-based and leisure-time physical activity per week were included in the study. The Ethics committee of Manchester Metropolitan University granted ethical approval and all experimental procedures complied with the Declaration of Helsinki.

Table 1 Participant physical characteristics according to *PTK2* genotype frequency for the rs7843014 and rs7460 polymorphisms

	All	rs7843014			<i>P</i>	rs7460			<i>P</i>
		AA	AC	CC		AA	AT	TT	
Frequency (%)	120 (100)	42 (35)	52 (43)	26 (22)		34 (28)	54 (45)	32 (27)	
Age (years)	20.6±2.3	20.4±2.7	20.7±2.3	20.8±2.4	0.705	20.4±2.3	20.8±2.4	20.4±2.2	0.623
Height (m)	1.79±0.06	1.78±0.06	1.79±0.07	1.79±0.06	0.594	1.79±0.07	1.80±0.05	1.77±0.07	0.217
Mass (kg)	75.0±10.0	72.9±9.3	75.4±10.4	77.4±10.0	0.180	74.9±11.0	76.8±9.3	72.0±9.7	0.106
BMI (kg m ⁻²)	23.4±2.7	23.0±2.5	23.4±2.7	24.1±3.1	0.248	23.5±3.0	23.7±2.7	22.8±2.5	0.326

Frequency data presented as count (%), all other data presented as mean±SD

Knee extension and flexion torque

Maximal voluntary isometric knee flexion (MVC_{KF}) torque and MVC_{KE} was assessed using an isokinetic dynamometer (Cybex Norm, Cybex International Inc., NY, USA) whilst participants were seated at 85° hip flexion and secured with straps across the shoulders, waist and right thigh. Participants completed a minimum of three MVC_{KE} and MVC_{KF} contractions at knee joint angles of 70°, 80° and 90° of knee flexion on the right leg in a randomised order, with 2 min rest between contractions. The knee joint angle at which peak MVC_{KE} torque occurred was considered the optimal angle and was used for subsequent measurements of net MVC_{KE} and specific force. The right limb of each participant was selected for assessment as previous investigations using untrained participants have reported no significant bilateral differences in MVC torque (Lindle et al. 1997; Hageman et al. 1988; Häkkinen et al. 1998), in addition to determinants of this, such as muscle architecture (Kearns et al. 2001), size and EMG (Häkkinen et al. 1996).

Muscle activation and co-activation

Two self-adhesive electrodes (7.5×12.5 cm; Tyco Galvanic Pad, Uni-Patch, MN, USA) were positioned over the muscle belly of the vastus medialis, rectus femoris and vastus lateralis and connected to an external stimulation device (DS7, Digitimer stimulator, Welwyn, Garden City, UK) such that the anode was placed 5–10 cm proximal to the superior border of the patella and the cathode was located 5–10 cm distal to the inguinal crease (Place et al. 2010). With the participant at rest, the maximal twitch torque stimulation intensity was identified by administering single twitches of increasing current intensity until no further increases in twitch torque were observed. The current intensity at which no further increases in twitch torque were observed was defined as the supramaximal stimulation intensity and this intensity was superimposed during MVC_{KE} to allow for the calculation of net MVC_{KE} torque, together with co-activation torque. Antagonist muscle co-activation during MVC_{KE} was determined through electromyographic (EMG)

assessment of the biceps femoris. Two pre-gelled Ag–AgCl electrodes (Ambu, Neuroline 720, Denmark) were positioned in the mid-sagittal plane over the distal third of the long head of the biceps femoris, with an inter-electrode distance of 20 mm, and a reference electrode was positioned over the lateral tibial condyle. The integral of the root mean square EMG activity corresponding to peak MVC_{KE} torque at the optimal knee joint angle was calculated and averaged over 0.5 s either side of the instantaneous peak. EMG activity of the biceps femoris during MVC_{KF} was measured and, assuming a linear relationship between torque and EMG activity, was used to estimate co-activation torque (Reeves et al. 2004b). Subsequently, net MVC_{KE} torque was calculated as $CcT + (MVC_{KE} + STT)$, where CcT is the co-activation torque and STT is the superimposed twitch torque obtained using the interpolated twitch technique.

Muscle architecture

Vastus lateralis (VL) muscle architecture was assessed in vivo at 50% of muscle length using B-mode ultrasonography (AU5, Esaote, Italy) during MVC_{KE} at the predetermined optimum joint angle. The 40 mm, 7.5 MHz linear-array probe was placed perpendicular to the skin over an echo-absorptive reference marker in the mid-sagittal plane during image acquisition. Ultrasound scans were recorded at a sampling frequency of 25 Hz in audio video interleave (AVI) format and all signals of torque, electrical stimuli and EMG activity were displayed on a computer screen, interfaced with an acquisition system (AcqKnowledge, Biopac Systems, Santa Barbara, USA), to enable analogue-to-digital conversion at a sampling frequency of 2000 Hz. Single images were captured for measurement of pennation angle and fascicle length on a minimum of three fascicles from which the mean was recorded (NIH ImageJ, version 1.44o, National Institute of Health, Bethesda, Maryland). Measurement of fascicle length in all instances required extrapolation of the superficial and deep aponeuroses to allow for estimation of fascicle length, due to fascicles extending beyond the ultrasound field of view (Reeves and Narici 2003).

Tendon moment arm length

Patellar tendon moment arm length was measured using a single, low-energy (0.9 μ Sv) sagittal plane dual energy X-ray absorptiometry (DXA) scan (22.6 \times 13.7 cm; Hologic Discovery W, Vertec Scientific Ltd, UK) (Erskine et al. 2014). During scanning, each participant lay on their side with the hip flexed at 85° and the right knee joint positioned at the previously determined optimum angle using a goniometer. Scans were exported (OsiriX 5.0.2, Pixmeo Sarl, Geneva, Switzerland) and the perpendicular distance between the tibiofemoral contact point and the axis of the patellar tendon was measured as the patellar tendon moment arm length (Tsaopoulos et al. 2006). Patellar tendon force was calculated by dividing net MVC_{KE} torque by patellar tendon moment arm length. The contribution of the VL to patellar tendon force was estimated using previously reported data (Narici et al. 1992), and divided by the cosine of pennation angle to estimate VL fascicle force.

VL muscle size and specific force

Transverse ultrasound scans were obtained from the medial to lateral muscle border at 50% of VL muscle length and recorded in AVI format to assess VL anatomical cross-sectional area (ACSA) (Reeves et al. 2004a). Single scans were contour matched to provide a complete image of VL ACSA, which was measured using digitizing software. Muscle volume was estimated using ACSA, VL muscle length and a series of regression-derived constants, as validated previously (Morse et al. 2007). Calculation of VL muscle PCSA was achieved by dividing VL muscle volume by fascicle length. Ultimately, VL specific force was calculated by dividing VL fascicle force by VL PCSA (Reeves et al. 2004b).

Blood sampling, DNA isolation and genotyping

A 5 mL blood sample was collected from a superficial forearm vein of 96 participants into EDTA tubes (BD Vacutainer Systems, Plymouth, UK) and stored at -20°C in 2 mL microcentrifuge tubes (Eppendorf AG, Hamburg, Germany). For 24 participants, buccal cell samples were obtained in duplicate (Whatman Sterile OmniSwab, GE Healthcare, USA) following a minimum 1-h abstinence from food and drink, according to the manufacturer's guidelines. Each collection tip was ejected into a 2 mL microcentrifuge tube and stored at -20°C .

The Qiagen QIAcube spin protocol (Qiagen, Crawley, UK) was completed in accordance with the manufacturer's guidelines using the buffers contained in the Qiagen DNA Blood Mini kit for the extraction of genomic DNA from whole blood and buccal samples. Genotyping of *PTK2*

rs7843014 and rs7460 was carried out using real-time PCR in 96-well plates each containing a reaction volume of 10 μ L. For DNA obtained from whole blood samples, the reaction volume contained 0.2 μ L of participant DNA (9.9 \pm 1.1 ng, amounts determined using ~20% of participant DNA samples), 5 μ L of TaqMan genotyping master mix (Applied Biosystems®, UK), 4.3 μ L of nuclease-free H₂O and 0.5 μ L of TaqMan genotyping assay mix. For DNA samples obtained from buccal cells, the 10 μ L reaction volume contained 1 μ L of participant DNA (18.6 \pm 4.6 ng), 5 μ L of TaqMan genotyping master mix, 3.5 μ L of nuclease-free H₂O and 0.5 μ L of TaqMan genotyping assay mix. In control wells, nuclease-free H₂O replaced the DNA sample.

The PCR plate was sealed (Microseal 'B' adhesive seal, Bio-Rad Laboratories, Hercules, CA) and DNA amplification (Chromo4 Real-Time PCR Detection System, Bio-Rad Laboratories) of each polymorphism was completed using the following PCR protocol: 10 min at 95°C followed by 40 cycles of denaturation for 15 s at 92°C, primer annealing and extension for 1 min at 60°C and plate read. Genotypes were determined by measurement of end-point fluorescence of VIC® and FAM® detected by the PCR machine and analysed using Opticon Monitor software (Bio-Rad Laboratories). For the rs7460 polymorphism the alleles indicated by VIC® and FAM® were converted and nucleotides were reported according to NCBI (<http://www.ncbi.nlm.nih.gov/snp/?term=rs7460>). All samples were analysed in duplicate and 100% agreement between all duplicate samples was achieved.

Statistical analysis

The frequency of each polymorphism was assessed for compliance with Hardy–Weinberg equilibrium using χ^2 tests. A one-way analysis of variance (ANOVA) was conducted to determine any significant differences in physical characteristics (height, mass, BMI and age) between genotypes. Pearson's correlations were used to identify variables that made a meaningful contribution to the variability of the muscle phenotype under investigation, and those identified were included as confounding variables in subsequent analyses of covariance (ANCOVA). ANOVA, or where appropriate ANCOVA, was conducted to investigate genotype differences in muscle strength phenotypes and linear trend analyses were used to assess genotype associations with muscle strength phenotypes. Post-hoc pairwise comparisons were subjected to Benjamini–Hochberg corrections. Where a tendency between genotype groups was observed (P between 0.05 and 0.10), the two genotype groups with most similar means were combined and compared using an independent samples t test (Erskine et al. 2012). Furthermore, the extent of linkage disequilibrium between *PTK2*

rs7843014 and rs7460 was determined using CubeX online software (<http://www.oege.org/software/cubex>) to estimate haplotype frequencies and calculate D' and R^2 as the difference between the observed and expected haplotype frequencies (Gaunt et al. 2007). Statistical analyses were performed using SPSS version 19.0 and alpha set at 0.05. Data are presented as mean \pm SD.

Results

Genotype frequencies for *PTK2* rs7843014 (AA=35.0%, AC=43.3%, CC=21.7%) and rs7460 (AA=28.3%, AT=45.0%, TT=26.7%) were in Hardy–Weinberg equilibrium ($\chi^2 \leq 1.661$, $P \geq 0.436$). There were no differences between genotypes of either SNP for height, mass, BMI or age ($P \geq 0.106$; Table 1). There were moderate correlations between muscle volume ($r \geq 0.361$, $P \leq 4.7 \times 10^{-4}$), PCSA ($r \geq 0.417$, $P \leq 2.2 \times 10^{-6}$) and all muscle strength phenotypes. Consequently, muscle volume was included as a covariate in analyses of MVC_{KE} torque and net MVC_{KE} torque but not specific force, as this is accounted for during the calculation of muscle specific force.

VL specific force tended to differ between genotype groups for *PTK2* rs7843014 ($P=0.060$) and rs7460 ($P=0.089$; Table 2). VL specific force of rs7843014 AA homozygotes was 8.3% higher than C-allele carriers ($P=0.017$; Fig. 1). Similarly, rs7460 AA homozygotes had 5.4% higher VL specific force than T-allele carriers ($P=0.029$; Fig. 1). Linkage disequilibrium between the two SNPs was calculated as $D' = 0.894$ and $R^2=0.591$. Thus, while individual contributions of rs7843014 and rs7460 to inter-individual variability in VL specific force were 3.5% ($P=0.041$) and 3.3% ($P=0.048$), respectively, no more variability (3.8%, $P=0.107$) could be accounted for by both SNPs together.

Net MVC_{KE} torque tended to differ between rs7843014 genotype groups ($P=0.094$; Table 2), although there was

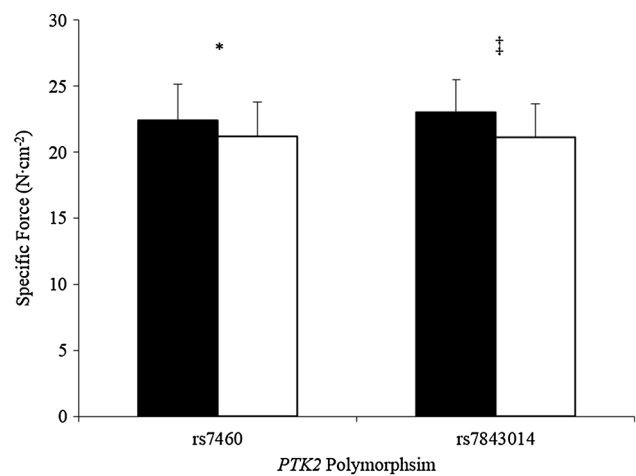


Fig. 1 Vastus lateralis specific force according to *PTK2* rs7843014 and rs7460 genotype. rs7460 AA homozygotes (black bars) had higher specific force than T-allele carriers (white bars; * $P=0.029$) and rs7843014 AA homozygotes (black bars) had higher specific force than C-allele carriers (white bars; ‡ $P=0.017$). Data are mean \pm SD

no difference between AA homozygotes and C-allele carriers ($P=0.128$). However, there was a linear trend in net MVC_{KE} torque of AA > AC > CC ($P=0.041$, $\eta_p^2=0.042$). No differences in net MVC_{KE} torque were observed between genotype groups for *PTK2* rs7460 ($P=0.275$), nor were there any genotype differences in MVC_{KE} torque for either polymorphism ($P \geq 0.107$; Table 2). Similarly, no differences in any architectural or size phenotypes were observed between genotype groups for either polymorphism ($P \geq 0.133$; Table 2).

Discussion

The aim of this study was to investigate the relationship between polymorphisms in the *PTK2* gene and muscle

Table 2 Muscle strength characteristics according to *PTK2* rs7843014 and rs7460 genotype

	rs7843014			<i>P</i>	rs7460			<i>P</i>
	AA	AC	CC		AA	AT	TT	
VL fascicle length (cm)	7.2 \pm 1.8	7.0 \pm 1.4	7.5 \pm 1.4	0.371	7.3 \pm 1.4	7.1 \pm 1.7	7.2 \pm 1.7	0.844
VL pennation angle (°)	19.2 \pm 3.8	20.1 \pm 4.0	18.5 \pm 3.6	0.183	18.8 \pm 3.1	20.2 \pm 4.6	18.8 \pm 3.0	0.133
VL ACSA (cm ²)	21.0 \pm 2.4	21.6 \pm 2.7	21.9 \pm 2.2	0.351	21.6 \pm 2.3	21.5 \pm 2.6	21.1 \pm 2.7	0.689
VL PCSA (cm ²)	71.3 \pm 10.9	71.6 \pm 13.6	72.7 \pm 13.8	0.912	70.4 \pm 15.0	72.3 \pm 11.5	72.2 \pm 12.3	0.768
Peak MVC_{KE} torque (N m)	260 \pm 50.9	250 \pm 43.0	243 \pm 44.7	0.107	253 \pm 51.7	250 \pm 42.0	243 \pm 45.9	0.495
Net MVC_{KE} torque (N m)	286 \pm 53.0	272 \pm 56.1	257 \pm 46.2	0.094	276 \pm 57.8	267 \pm 53.8	259 \pm 46.9	0.275
VL specific force (N cm ⁻²)	22.3 \pm 2.6	21.1 \pm 2.6	21.1 \pm 2.6	0.060	22.4 \pm 2.8	21.3 \pm 2.5	21.1 \pm 2.6	0.089

P denotes genotype group differences

VL vastus lateralis, ACSA anatomical cross-sectional area, PCSA physiological cross-sectional area, MVC_{KE} maximal voluntary knee extension

strength phenotypes in a healthy population. Genotype and allele frequencies for *PTK2* rs7843014 and rs7460 were similar to previous reports in Caucasian populations (Erskine et al. 2012), while mean MVC_{KE} torque, net MVC_{KE} torque and VL specific force in the current study (250 N m, 274 N m and 21.4 N cm^{-2} , respectively) were in accordance with previous reports in comparable populations (Narici et al. 1992; Erskine et al. 2012, 2009). We report a genotype–phenotype association between each of the *PTK2* SNPs and VL specific force.

PTK2 is the gene encoding FAK, a protein integral to the formation and turnover of muscle costameres (Quach and Rando 2006). During muscle contraction, muscle costamere complexes are involved in transmitting force laterally from the muscle contractile elements to the ECM (Bloch and Gonzalez-Serratos 2003). Differing ability of muscle to transmit force laterally may translate into variability in maximal joint torque and/or muscle specific force. In the current study, polymorphisms in the *PTK2* gene were associated with VL specific force but not MVC_{KE} torque, net MVC_{KE} torque, muscle architecture or size. Individuals homozygous for the rs7843014 A-allele had greater VL specific force than their C-allele counterparts (8.3%), and those homozygous for the rs7460 A-allele demonstrated greater VL specific force than T-allele carriers (5.4%; Fig. 1). When considering a previous study observed no differences in quadriceps femoris specific force between men, women, boys or girls (O'Brien et al. 2010) these genotype-group observations represent important differences. Furthermore, the inter-individual variability observed within this phenotype was 16%, and together, the two *PTK2* SNPs explain a substantial proportion (~25%) of this variability. A previous report on the influence of *PTK2* rs7843014 and rs7460 on muscle strength also reported a significant association between quadriceps femoris muscle specific force and A-allele (rs7843014 and rs7460) homozygotes in a smaller Caucasian cohort (Erskine et al. 2012). Thus, the current investigation provides the first independent replication of the findings from this previous smaller cohort study and together these findings demonstrate a probable association of *PTK2* with muscle specific force.

A plausible explanation for the findings of the current investigation may be that AA (rs7843014 and rs7460) homozygotes experience altered *PTK2* expression compared to their C-allele (rs7843014) and T-allele (rs7460) carrying counterparts. Interestingly, an association between CC (rs7843014) and TT (rs7460) genotypes and lower gene expression in Spanish Caucasians has been reported (Garatachea et al. 2014). Furthermore, *in vitro* FAK-null cells demonstrate diminished integrin activation and a reduced adhesion-strengthening rate, which results in the formation of fewer integrin-ECM bonds, compared to cells expressing FAK (Michael et al. 2009). Contrastingly, however,

evidence exists reporting FAK-null cells actually form stronger adhesions, possess enhanced contractile properties and migrate slower than their wild-type counterparts (Ilic et al. 1995; Chen et al. 2002; Ren et al. 2000), thus demonstrating the complexity surrounding FAK-regulated adhesion strength and the role of *PTK2* in the variation within muscle specific force production. Both rs7843014 and rs7460 are in non-coding regions, and although it is unlikely that either the SNP would result in changes to the amino acid sequence of FAK that would elicit comparable effects to those of FAK-null cells, potential alterations to the magnitude, location and timing of gene expression, or mRNA stability as a consequence of one or both of these SNPs cannot be discounted (Laguette et al. 2011; September et al. 2009).

The observed association between the AA genotypes and enhanced VL specific force might be attributable to an increased costamere density as a consequence of more integrin-ECM bonds with higher gene expression (Bang et al. 2001), compared to T-allele (rs7460) and C-allele (rs7843014) carriers (Garatachea et al. 2014). Although it remains unclear how an increased costamere density corresponds to an increase in specific force, it could be achieved via enhanced lateral transmission of force (Jones et al. 1989; Street 1983). An increased costamere density is likely to result in more intermediate connections between the ECM, myofibres and aponeurosis, effectively splitting serial sarcomeres into numerous force-generating units whilst also reducing functional muscle fibre length (Jones et al. 1989). Assuming all else is equal, it is hypothesized that more force-generating units within a fibre would reduce maximal shortening velocity whilst enabling a simultaneous increase in force generation within a given PCSA, thus resulting in increased muscle specific force (Jones et al. 1989; Huijij 1999). Whilst evidence to support this hypothesis is tenuous, Erskine et al. (2011) observed an increase in specific force but not power per unit of muscle mass following resistance training. Given that maximal muscle power is the product of maximal shortening velocity, which is expected to reduce with an increased costamere density, and maximal force, which is expected to increase with an increased costamere density, when power is normalized to muscle volume it should remain unchanged despite an increase in specific force as a consequence of an increased costamere density. As Erskine et al. (2011) did not determine maximal shortening velocity, it is unclear if their observed increase in specific force was inversely proportional to maximal shortening velocity and would explain why normalized power remained unchanged. Future research is, therefore, necessary to include measurements of maximal shortening velocity alongside specific force and power, to confirm or refute this speculation. Although the mechanism by which costamere density might contribute to enhanced lateral

force transmission is currently undetermined, proposed mechanisms include the presence of a greater number of costameres per muscle fibre and/or a greater number of smaller fibres per muscle and thus a higher fibre perimeter-to-area ratio (Erskine et al. 2012). If the latter were true, a muscle containing a greater number of smaller fibres could exhibit comparable muscle size and architecture to a muscle containing fewer larger fibres. Accordingly, the data presented in Table 2 revealed no associations between *PTK2* genotype and any muscle architectural or size phenotypes, thus suggesting a greater number of smaller fibres may contribute to an increased costamere density. Future research should investigate *PTK2* genotype differences in muscle costamere density via direct measurement to confirm or refute this hypothesis.

Further to specific force, we hypothesized that *PTK2* genotype would be associated with MVC_{KE} and net MVC_{KE} torque, but no associations were observed. It is possible that any genotype associations with these phenotypes were masked by inter-individual variability in other important determinants of torque, such as muscle size, architecture, activation capacity, co-activation and tendon moment arm length (Stebbins et al. 2014). Indeed the inter-individual variability observed in these determinants within the current study ranged from 6% for muscle activation and co-activation capacity to ~20% for muscle architecture and size.

Conclusion

This study has identified an association between polymorphisms in the *PTK2* gene and VL muscle specific force, thus providing an independent replication of previous research conducted on the quadriceps femoris muscle group (Erskine et al. 2012). These findings extend our understanding of the influence of genetic variation on the intrinsic strength of a muscle and have potential implications for ageing populations for whom the maintenance of muscle specific force is important to promote independence and a better quality of life.

Compliance with ethical standards

Conflict of interest The authors declare they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institution and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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