Do PTK2 gene polymorphisms contribute to the inter-individual variability in muscle strength and the response to resistance training? A preliminary report

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ABSTRACT

The protein tyrosine kinase-2 (PTK2) gene encodes focal adhesion kinase, a structural protein involved in lateral transmission of muscle fiber force. We investigated whether single nucleotide polymorphisms (SNPs) of the PTK2 gene were associated with various indices of human skeletal muscle strength and the inter-individual variability in the strength responses to resistance training. We determined unilateral knee extension single repetition maximum (1-RM), maximum isometric voluntary contraction (MVC) knee joint torque and quadriceps femoris muscle specific force (maximum force per muscle physiological cross-sectional area), before and after 9-weeks of knee extension resistance training in 51 untrained young men. All participants were genotyped for the PTK2 intronic rs7843014 A/C and 3' UTR rs7460 A/T SNPs. There were no genotype associations with baseline measures or post-training changes in 1-RM or MVC. Although the training-induced increase in specific force was similar for all PTK2 genotypes, baseline specific force was higher in PTK2 rs7843014 AA and rs7460 TT homozygotes than in their respective rs7843014 C- ($P = 0.016$) and rs7460 A-allele ($P = 0.009$) carriers. These associations between muscle specific force and PTK2 SNPs suggest that inter-individual differences exist in the way force is transmitted from the muscle fibers to the tendon. Therefore, our results demonstrate for the first time the impact of genetic variation on the intrinsic strength of human skeletal muscle.

Key words: Protein tyrosine kinase-2 (PTK2); focal adhesion kinase (FAK); gene polymorphisms; costameres; lateral force transmission.

INTRODUCTION

Muscle force is transmitted to the tendon along the length of a muscle fiber and also laterally via attachments to the surrounding matrix of connective tissue (27). It has been suggested that an increase in lateral attachments after resistance training might result in an enhanced muscle specific force [maximum force per physiological cross-sectional area (PCSA)] (7, 12). Such attachments have been identified as intra-sarcolemmal protein complexes known as "costameres" (19), which are associated with the lateral transmission of muscle fiber force (6). Thus, costameres could enable each muscle fiber to act as multiple force-generating units, thus increasing the specific force of the whole muscle.

Mechanical tension is essential in regulating costameric protein expression (29) and resistance training is known to modulate the expression of costameric proteins, such as desmin (32), alpha-1-syntrophin and dystrophin (14) in humans, while focal adhesion kinase (FAK) and paxillin expression and activity are increased in stretch-induced hypertrophied rooster skeletal muscle (11). The integrin-associated tyrosine kinase, FAK, has been shown to play a major role in costamere formation and turnover (4, 20) and FAK expression is controlled at the level of the protein tyrosine kinase-2 (PTK2) gene. Therefore, polymorphisms of the PTK2 gene could potentially underpin the considerable inter-individual variability reported in untrained human muscle specific force [ranging from 22 to 40 $N \cdot cm^{-2}$ (8)], and in the training-induced relative change in specific force, which varies between -5% and +39% (9).

As muscle strength and training responses are important from a clinical perspective, e.g. the response to rehabilitation following injury, we aimed to elucidate whether

single nucleotide polymorphisms (SNPs) of the PTK2 gene were associated with *in vivo* muscle specific force and functional measures of strength, both before and after resistance training. We hypothesized that two PTK2 SNPs (the intronic rs7843014 and the 3' UTR rs7460 SNP) would be associated with QF muscle specific force and with the change in specific force following training.

MATERIALS AND METHODS

Participants

Fifty-one untrained Caucasian males, aged 20.3 ± 3.1 years, height 178.1 ± 5.6 cm, body mass 75.4 ± 10.6 kg, body mass index (BMI) 23.7 ± 2.6 (mean ± SD) provided written informed consent prior to their involvement in the study, which complied with the Declaration of Helsinki and was approved by the local ethics committee of the Manchester Metropolitan University. Study volunteers were excluded if their age was outside the range of 18-39 years, they had a history of lower-limb fracture, had taken part in strength training within the 12 months prior to the study, had used dietary supplements or performance enhancing aids, or if they were considered to be in ill health (determined by their responses to a health questionnaire). Participants were familiarized with all test procedures and equipment within a 14-day period prior to the baseline measurements. Phenotype data from these participants have been reported previously (9).

Habitual physical activity rating

The habitual physical activity rating (PAR) of each participant was assessed by questionnaire (2) immediately prior to the training period. The overall PAR was scored using a scale from 1 to 5 points, where 1 was the least active, 3 was

intermediate and 5 was extremely active. Participants were asked to maintain their PAR and habitual dietary intake over the course of the study.

Experimental design

Maximum patellar tendon force, QF muscle volume, physiological cross-sectional area (PCSA) and specific force were determined in the right limb [as described in Method 2 of (8)] before and after nine weeks of high-intensity unilateral knee extension resistance training (10) in 51 previously untrained men. In addition, all participants had blood samples isolated, which were genotyped for the PTK2 rs7460 A/T and rs7843014 A/C SNPs.

Progressive resistance training

The supervised resistance training protocol has been described in detail elsewhere (10). Briefly, supervised knee extension training was performed unilaterally three times per week for nine weeks. The maximum training load that could be lifted once only (1-RM) throughout the full range of knee extension (110° to 20° of knee flexion; 0° = full knee extension) was assessed at the beginning of the training program and re-evaluated at the start of each week on a standard knee extension machine (Technogym, Gambettola, Italy). The training intensity was set in relation to the 1-RM and was therefore progressively increased throughout the nine weeks of training. Each session comprised a warm-up set of 10 knee extension repetitions at 40% of the revised 1-RM, followed by four sets (2 min rest between each) of 10 repetitions at 80% 1-RM. Compliance with the training protocol was 100%, with each participant completing all 27 training sessions.

Maximum patellar tendon force

The method used to assess maximum patellar tendon force has been explained in detail elsewhere (8). In summary, participants performed isometric knee extension maximal voluntary contractions (MVCs) on a dynamometer (Cybex Norm, Cybex International, Ronkonkoma, NY) at optimum knee joint angle, which ranged from 70-90° knee flexion. Participants were seated with a hip angle of 85° (supine = 180°) and were fixed with inextensible straps to the strength-testing chair. Co-contraction torque of the antagonist muscles during knee extension MVC was calculated by comparing electromyographic activity of the biceps femoris muscle during maximal isometric knee extension and maximal isometric knee flexion (21). Two bipolar silver chloride surface electrodes (Neuroline, Medicotest, Rugmarken, Denmark) were placed 20 mm apart along the sagittal axis over the muscle belly (the location was recorded on an acetate for further tests) and one reference electrode was positioned over the lateral tibial condyle. The root mean square of the raw EMG signal was calculated over one second around the peak torque during each maximum voluntary isometric knee extension and flexion at optimum joint angle and the torque produced by the hamstrings during knee extension was estimated assuming a linear relationship between torque and EMG activity (21). The estimated antagonist torque obtained at the optimum knee extension joint angle was used to calculate the maximum overall knee extension torque. Voluntary QF muscle activation was assessed using the interpolated twitch technique (25), whereby the participant received a supramaximal twitch (Digitimer stimulator model DS7, Welwyn Garden City, UK) via two 7.5 cm x 12.5 cm self-adhesive electrodes (Versastim, Conmed, New York, NY) placed distally (anode) and proximally (cathode) over the QF muscle, once before MVC (control twitch) and once during MVC. True maximum torque (TMT) was calculated as:

$$TMT = MVC(C) \cdot (1-t/T)^{-1}$$

where t is the amplitude of the superimposed twitch, T is the value of the twitch before the MVC and MVC(C) is MVC corrected for antagonist muscle co-activation. The percentage of voluntary muscle activation was given by:

$$100 \cdot (1-t/T)$$

The patellar tendon moment arm (d_{PT}) was determined using a 0.2-T magnetic resonance imaging (MRI) scanner (G-Scan, Esaote Biomedica, Genoa, Italy), as previously described (30). Sagittal and coronal-plane knee scans were acquired using a Turbo 3D T1-weighted sequence with the following scanning parameters: time of repetition 40 ms; time to echo 16 ms; matrix 256 x 256; field of view 180 mm x 180 mm; slice thickness 3.4 mm; interslice gap 0 mm. The knee was scanned at rest with the participant in the supine position and the knee fully extended. Coronal scans were used to identify the appropriate sagittal scans, which were used to locate the centre of rotation (COR), i.e. the midpoint of the shortest distance between the two femoral condyles and the tibial plateau, and d_{PT} was defined as the perpendicular distance between the COR and the axis of the patellar tendon (30). Previously reported ratios of d_{PT} at full extension (0 degrees knee flexion) to d_{PT} at of 70, 80 and 90 degrees knee flexion (3) were used to calculate d_{PT} at optimum knee joint angle in this study. Subsequently, maximum force resolved at the patellar tendon (F_t) was calculated as:

$$F_t = TMT / d_{PT}$$

Muscle physiological cross-sectional area (PCSA)

QF muscle PCSA was determined from a method that has been described in detail previously [Method 2 of (8)]. In brief, ultrasonography (MyLab25, Esaote Biomedica, Genoa, Italy) was used to identify femur length (the distance from the proximal origin

of the VL muscle to the tibiofemoral contact point). ACSA of each component QF muscle was assessed from transverse MRI scans acquired at 40% femur length from the distal end. QF muscle volume (V_m) was calculated by adapting a previously described method (15) that incorporated femur length, the ACSA of each constituent QF muscle and a series of regression equations. VL muscle fascicle length (L_f) and pennation angle (θ_p) were measured during knee extension MVC at optimum knee angle using ultrasonography at 50% of the muscle length along the mid-sagittal plane. Dividing V_m by VL muscle L_f provided QF PCSA [VL L_f has been shown to be representative of the L_f for the whole QF muscle group (8)].

In vivo muscle specific force

QF muscle force is reduced when resolved along the patellar tendon according to the θ_p. Therefore, QF PCSA was multiplied by the cosine of VL θ_p, which provided the reduced QF PCSA. Consequently, specific force was determined by dividing F_t by the reduced QF PCSA (8).

Blood sampling

A 10-mL blood sample was drawn into 10-mL EDTA tubes (BD Vacutainer Systems, Plymouth, UK) from a superficial forearm vein. The whole blood was aliquotted into 2-mL tubes (Eppendorf AG, Hamburg, Germany) and stored at -80°C until subsequent analysis.

DNA extraction and determination of PTK2 genotype

Automated DNA extraction was performed using a QIAcube (Qiagen, Crawley, UK) in association with the QIAamp DNA Blood Kit (Qiagen, Crawley, UK), and following the QIAamp spin protocol for DNA purification from whole blood.

Real-time polymerase chain reaction (PCR) was performed to determine the genotype of the PTK2 polymorphisms in each participant. Reactions were carried out on 96-well microtiter plates. Each 10-µL reaction volume contained: 5-µL Genotyping Master Mix (Applied Biosystems, Foster City, CA), 4.3-µL nuclease-free H_2O (Qiagen, Crawley, UK), 0.5-µL genotyping assay mix (Applied Biosystems, Foster City, CA), plus 0.2-µL sample DNA at a concentration of ~30 $ng \cdot \mu L^{-1}$ and an A260/A280 ratio of 1.7–1.9. TaqMan rs7843014 and rs7460 SNP genotyping assay mixes were used, and each mix included the appropriate TaqMan primers and probes.

For control wells, 0.2-µL nuclease-free H_2O replaced the DNA template. Following sealing (Microseal 'B' adhesive seal, BioRad Laboratories, Hercules, CA) and centrifugation at 8,000 RPM for 1 min, DNA amplification (Chromo4 Real-Time PCR Detection System, BioRad Laboratories, Hercules, CA) was performed using the following PCR protocol: denaturation at 95°C for 10 min, followed by 40 cycles of incubation at 92°C for 15 s then annealing and extension at 60°C for 1 min. PTK2 genotypes were ultimately determined using Opticon Monitor 3.1 software (BioRad Laboratories, Hercules, CA). All samples were analyzed in duplicate and in all cases there was 100% agreement between genotype for samples from the same participant.

We performed the genotyping in accordance with published genotyping and quality control recommendations (5). These included describing genotyping assays and

protocols in detail, producing an overview of sample ID and well number prior to genotyping, including external control samples, incorporating internal controls by genotyping samples in duplicate (from the same DNA collection), comparing current genotype frequencies with previously published frequencies in a similar population and evaluating the level of agreement with the Hardy-Weinberg principle. The extent of linkage disequilibrium (LD) between the two PTK2 SNPs was investigated by using freely available software (http://linkage.rockefeller.edu/ott/eh.htm) to estimate the haplotype frequencies. The difference between the expected and observed haplotype frequencies was then calculated and reported as D' and R^2.

Statistical analysis

Genotype frequencies for each PTK2 SNP were tested for compliance with the Hardy-Weinberg principle using χ^2 tests. Repeated measures ANOVAs [within subjects factor: time (pre- and post-training); between subjects factor: group (3 genotype levels)] were used to detect associations between each PTK2 SNP and 1-RM, MVC knee joint torque and QF muscle specific force before and after training. If a tendency was observed between group or for a group x time interaction, i.e. $0.05 < P < 0.10$, the two genotypes with similar means were pooled and the ANOVA re-run with post-hoc independent *t*-tests. The individual and combined contributions of the PTK2 SNPs towards the inter-individual variance in muscle specific force were determined using a multiple linear regression model that included both SNPs. Significance was accepted when $P < 0.05$ and statistical tests were performed using SPSS v19. All data are presented as mean ± standard deviation (SD) unless otherwise stated.

RESULTS

PTK2 genotypes

The genotype frequencies for the PTK2 rs7843014 (AA = 37.3%; AC = 41.2%; CC = 21.6%) and rs7460 (AA = 25.5%; AT = 41.2%; TT = 33.3%) polymorphisms were all in Hardy-Weinberg equilibrium ($P \geq 0.473$). Further, the PTK2 rs7843014 A/C and rs7460 A/T allele frequencies were similar to those reported elsewhere for Caucasian populations (31).

Habitual physical activity rating

The habitual physical activity rating (PAR) for the total cohort was 2.7 ± 0.3 and can be described as slightly less than "intermediate" (2). Furthermore, none of the physical characteristics (age, stature, body mass, BMI) or PAR differed between genotype regarding either polymorphism: PTK2 rs7843014 A/C ($P \geq 0.135$); rs7460 A/T ($P \geq 0.102$).

Single repetition maximum (1-RM)

Baseline 1-RM (54.3 ± 11.0 kg for the whole cohort) did not differ between genotype for both the rs7843014 (ANOVA, genotype $P = 0.659$; Table 1) and the rs7460 (ANOVA, genotype $P = 0.740$; Table 1) SNPs. Similarly, the % change in 1-RM (+66.8 ± 30.2% for the entire group) did not differ between genotype for either SNP (rs7843014: ANOVA, time x genotype $P = 0.306$; Table 1; rs7460: ANOVA, time x genotype $P = 0.839$; Table 2).

Table 1 near here.

Maximum isometric voluntary contraction (MVC) knee joint torque

Before training, MVC torque (248 ± 52 N·m for the entire cohort) did not differ between genotype regarding either the rs7843014 (ANOVA, genotype $P = 0.826$; Table 1) or the rs7460 (ANOVA, genotype $P = 0.697$; Table 2) SNPs. In addition, the % change in MVC torque (26.1 ± 10.7% for the whole group) did not differ between genotype for either SNP (rs7843014: ANOVA, time x genotype $P = 0.642$; Table 1; rs7460: ANOVA, time x genotype $P = 0.553$; Table 2).

Table 2 near here.

Muscle physiological cross-sectional area (PCSA)

Prior to training, QF muscle PCSA for the total cohort was 239 ± 40 cm^2, and there was no association with either SNP (ANOVA, genotype $P \geq 0.314$). Nine weeks of resistance training led to a 5.8 ± 4.5% increase in muscle PCSA (ANOVA, time $P < 0.0005$), which was independent of PTK2 genotype (ANOVA, time x genotype $P \geq 0.963$).

Muscle specific force

Regarding untrained muscle specific force (25.5 ± 5.2 $N \cdot cm^{-1}$ for the entire group), there were non-significant tendencies for PTK2 rs7843014 AA homozygotes to produce higher muscle specific force than their AC and CC counterparts (ANOVA genotype $P = 0.078$; Table 1), and the muscles of PTK2 rs7460 TT homozygotes to have higher specific force than AA and AT genotypes (ANOVA, genotype $P = 0.058$; Table 2). When the PTK2 rs7843014 AC and CC genotypes were pooled, the QF muscles of individuals homozygous for the A-allele expressed higher specific force than carriers of the C-allele before training (ANOVA, genotype $P = 0.023$; Table 1; *t*-

test $P = 0.016$; Fig. 1). Similarly, when the PTK2 rs7460 AA and AT genotypes were combined, QF muscle specific force was found to be higher in TT homozygotes than in A-allele carriers before training (ANOVA, genotype $P = 0.017$; Table 2; *t*-test $P = 0.009$; Fig. 1). However, there was no significant interaction between training and PTK2 genotype concerning QF muscle specific force and both the rs7843014 (ANOVA, time x genotype $P = 0.601$; time $P < 0.0005$; Table 1) and rs7460 (ANOVA, time x genotype $P = 0.461$; time $P < 0.0005$; Table 2) PTK2 SNPs, implying that specific force increased similarly among all three genotypes of both SNPs (16.4 ± 11.2% for the whole cohort).

Fig. 1 near here

As both SNPs of the PTK2 gene were associated with QF muscle specific force, and a large proportion of participants (33%) possessed both 'preferential' genotypes, it was further investigated whether or not the loci and PTK2 alleles were independent from each other. The estimated haplotype frequencies are presented in Table 3, and the deviation of the observed haplotype frequency from the expected frequency was calculated and defined as the linkage disequilibrium (LD). The LD for the two PTK2 polymorphisms was $D' = 0.905$ and $R^2 = 0.700$, which suggests that the two polymorphisms are in LD and are not completely independent from one another.

Table 3 near here.

Both PTK2 SNPs were associated with untrained muscle specific force, therefore the contribution of each SNP to the inter-individual variance in the respective muscle

297 phenotype was investigated. On an individual basis, PTK2 rs7843014 genotype
298 correlated with baseline muscle specific force (R^2 = 0.091; P = 0.031), suggesting that
299 genotype for this SNP alone contributed to ~9% of the inter-individual variability in
300 muscle specific force in the untrained state. PTK2 rs7460 genotype also correlated
301 with baseline muscle specific force (R^2 = 0.102; P = 0.022), thus implying that
302 genotype for this SNP explained ~10% of the inter-individual variability in untrained
303 muscle specific force. Combining the two PTK2 SNPs in a multiple regression model
304 led to a tendency towards a correlation with untrained muscle specific force (R^2 =
305 0.105; P = 0.071). Although this correlation did not reach statistical significance, it is
306 interesting to note that the coefficient of determination was similar to that of the
307 individual PTK2 SNPs, which is probably due to the relatively high LD between the
308 two SNPs.
309
310 **DISCUSSION**
311 We investigated whether associations existed between polymorphisms of the PTK2
312 gene and human skeletal muscle strength phenotypes before and after resistance
313 training. The two PTK2 gene polymorphisms were significantly associated with the
314 inter-individual variability in muscle specific force but did not contribute to the
315 observed inter-individual variation in the training response. Thus, our results highlight
316 a novel association between sequence variations in the PTK2 gene and the intrinsic
317 force generating capacity of human skeletal muscle, possibly via influences on lateral
318 force transmission. It should be noted, however, that the data presented in this study
319 are preliminary in that the sample size is a limitation. Thus, future studies should
320 attempt to replicate our findings using larger cohorts from the same and other ethnic

populations, which would increase both the power of the study and the confidence in our results.

The genotype frequencies for the PTK2 rs7843014 (AA = 37%; AC = 41%; CC = 22%) and rs7460 (AA = 26%; AT = 41%; TT = 33%) SNPs observed in our study were comparable to those reported previously for Caucasian populations (31). Baseline values for our entire cohort were similar to those reported elsewhere for this population concerning 1-RM lifting strength (13), isometric MVC knee joint torque (18), QF muscle PCSA (16) and specific force (16). Our observed 67% increase in 1-RM for the whole cohort was higher than some (22), but less than other (23, 24) reports of 1-RM strength gains following a similar period of knee extensor strength training. The 26% increase in isometric knee extensor MVC strength was less than some (26), but greater than other (1, 17) previously reported gains in isometric strength following a similar duration of knee extensor training. Regarding muscle hypertrophy, our observed 6% increase in QF muscle PCSA was comparable to previous reports of QF muscle size gains following resistance training of similar type and duration (1, 17). The 16% increase in muscle specific force was also comparable to that reported elsewhere following resistance training of the QF muscle, although in older individuals (21).

Focal adhesion kinase (FAK) plays an integral role in the costamere protein complex (4, 20) that is involved in the lateral transmission of force (6). As FAK is encoded by the PTK2 gene, we hypothesized that polymorphisms of this gene would explain part of the inter-individual variability in QF muscle specific force between untrained young men. We determined that individuals homozygous for the rs7843014 A-allele

had a higher muscle specific force than carriers of the C-allele, while QF muscle specific force was greater in rs7460 TT homozygotes compared to their A-allele counterparts.

Of the 19 participants who possessed one or both of the preferential PTK2 genotypes (rs7843014 AA or rs7460 TT), 17 people possessed both genotypes. Individually and combined, these two SNPs explained ~10% of the inter-individual variability in muscle specific force in the untrained state. Thus, these findings suggest that the two SNPs are not independently associated with *in vivo* muscle specific force but that they are in linkage disequilibrium, which is supported by a *D*' value of 0.91 and R^2 value of 0.70. This opens up several theoretical possibilities: 1) only one locus is functionally important regarding muscle specific force; 2) the SNPs become functional only when they occur together; 3) neither SNP influences muscle specific force but both are in linkage disequilibrium with the true functional variant that was not genotyped. In any case, neither of the PTK2 SNPs investigated in our study are of a kind likely to influence the amino acid sequence of the protein product. However, an alteration in DNA sequence in the 3'UTR region of a gene (e.g. the PTK2 rs7460 A/T polymorphism) has the potential to alter the level, location or timing of gene expression, while intronic genomic variants (e.g. the PTK2 rs7843014 A/C polymorphism) generally have the potential to influence gene expression and mRNA stability (28). Therefore, a potential influence of PTK2 gene polymorphisms on the concentration and time course of FAK expression warrants future investigation.

We hypothesized that PTK2 genotype would influence muscle specific force, leading to associations with functional measures of strength, such as maximum dynamic

lifting strength (1-RM) and isometric MVC knee joint torque. While we did find PTK2 genotype associations with untrained QF muscle specific force, we observed no association with baseline 1-RM or MVC torque. Although the intrinsic strength of the muscle undoubtedly contributes to both 1-RM and MVC torque, extrinsic factors such as neural drive, moment arm length, muscle size and architecture are also known to influence such strength measures independent of specific force (8), thus potentially masking any genotype associations with 1-RM and MVC torque.

Mechanical tension is known to regulate costameric protein expression (29) and resistance training increases the expression of costameric proteins, such as desmin (32), alpha-1-syntrophin and dystrophin (14) in humans, and FAK in hypertrophied rooster skeletal muscle (11). Therefore, we hypothesized that PTK2 genotype would influence the previously reported inter-individual variability in the training-induced change in muscle specific force, 1-RM and MVC torque (9), possibly through a genotype-dependent change in costameric density with loading. However, we found no association between either PTK2 SNP and the relative changes in muscle specific force, 1-RM or MVC torque following 9 weeks of resistance training. If any inherent difference between PTK2 genotype in the level of FAK protein expression is not preferentially enhanced with loading, muscle specific force will increase similarly between genotype. The higher muscle specific force at baseline might then be attributable to a greater muscle costameric density, which could be realized by 1) a higher number of costameres per muscle fiber perimeter and/or 2) a larger number of smaller fibers per muscle with a higher fiber perimeter to area ratio. Preliminary (unpublished) histological data from our laboratory suggest that people with the 'preferential' PTK2 AA genotype do have smaller muscle fiber CSAs than their 'non-

preferential' genotype counterparts, and together with a non-association between PTK2 genotype and muscle PCSA reported here, this would support the second hypothesis. In this case, a larger loading-induced increase in FAK expression in people with the higher baseline specific force, i.e. people with the 'preferential' PTK2 genotypes, might be offset by a relatively greater loading-induced increase in the perimeter of large compared to small fibers (assuming a similar relative increase in fiber CSA). This would lead to a similar increase in total muscle costameric density between genotype, which in turn would lead to comparable training-induced increases in muscle specific force.

Summary and conclusions

The inter-individual variability in QF muscle specific force can be partly explained by polymorphisms of the PTK2 gene that encodes FAK, a structural protein involved in the lateral transmission of muscle fiber force. Future experiments should investigate potential associations between PTK2 genotype and FAK expression in skeletal muscle. These results highlight the impact of genetic variation on the intrinsic strength of human skeletal muscle.

REFERENCES

1. **Aagaard P, Andersen JL, Dyhre-Poulsen P, Leffers AM, Wagner A, Magnusson SP, Halkjaer-Kristensen J, and Simonsen EB**. A mechanism for increased contractile strength of human pennate muscle in response to strength training: changes in muscle architecture. *J Physiol* 534: 613-623, 2001.

2. **Baecke JA, Burema J, and Frijters JE**. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. *Am J Clin Nutr* 36: 936-942, 1982.

3. **Baltzopoulos V**. A videofluoroscopy method for optical distortion correction and measurement of knee-joint kinematics. *Clin Biomech (Bristol, Avon)* 10: 85-92, 1995.

4. **Cary LA, and Guan JL**. Focal adhesion kinase in integrin-mediated signaling. *Front Biosci* 4: D102-113, 1999.

5. **Chanock SJ, Manolio T, Boehnke M, Boerwinkle E, Hunter DJ, Thomas G, Hirschhorn JN, Abecasis G, Altshuler D, Bailey-Wilson JE, Brooks LD, Cardon LR, Daly M, Donnelly P, Fraumeni JF, Jr., Freimer NB, Gerhard DS, Gunter C, Guttmacher AE, Guyer MS, Harris EL, Hoh J, Hoover R, Kong CA, Merikangas KR, Morton CC, Palmer LJ, Phimister EG, Rice JP, Roberts J, Rotimi C, Tucker MA, Vogan KJ, Wacholder S, Wijsman EM, Winn DM, and Collins FS**. Replicating genotype-phenotype associations. *Nature* 447: 655-660, 2007.

6. **Danowski BA, Imanaka-Yoshida K, Sanger JM, and Sanger JW**. Costameres are sites of force transmission to the substratum in adult rat cardiomyocytes. *J Cell Biol* 118: 1411-1420, 1992.

7. **Erskine RM, Jones DA, Maffulli N, Williams AG, Stewart CE, and Degens H**. What causes in vivo muscle specific tension to increase following resistance training? *Exp Physiol* 96: 145-155, 2011.

8. **Erskine RM, Jones DA, Maganaris CN, and Degens H**. In vivo specific tension of the human quadriceps femoris muscle. *Eur J Appl Physiol* 106: 827-838, 2009.

9. **Erskine RM, Jones DA, Williams AG, Stewart CE, and Degens H**. Inter-individual variability in the adaptation of human muscle specific tension to progressive resistance training. *Eur J Appl Physiol* 110: 1117-1125, 2010.

10. **Erskine RM, Jones DA, Williams AG, Stewart CE, and Degens H**. Resistance training increases in vivo quadriceps femoris muscle specific tension in young men. *Acta Physiol (Oxf)* 199: 83-89, 2010.

11. **Fluck M, Carson JA, Gordon SE, Ziemiecki A, and Booth FW**. Focal adhesion proteins FAK and paxillin increase in hypertrophied skeletal muscle. *Am J Physiol* 277: C152-162, 1999.

12. **Jones DA, Rutherford OM, and Parker DF**. Physiological changes in skeletal muscle as a result of strength training. *Q J Exp Physiol* 74: 233-256, 1989.

13. **Kokkonen J, Nelson AG, Eldredge C, and Winchester JB**. Chronic static stretching improves exercise performance. *Med Sci Sports Exerc* 39: 1825-1831, 2007.

14. **Kosek DJ, and Bamman MM**. Modulation of the dystrophin-associated protein complex in response to resistance training in young and older men. *J Appl Physiol* 104: 1476-1484, 2008.

15. **Morse CI, Degens H, and Jones DA**. The validity of estimating quadriceps volume from single MRI cross-sections in young men. *Eur J Appl Physiol* 100: 267-274, 2007.

16. **Narici MV, Landoni L, and Minetti AE**. Assessment of human knee extensor muscles stress from in vivo physiological cross-sectional area and strength measurements. *Eur J Appl Physiol Occup Physiol* 65: 438-444, 1992.

17. **Narici MV, Roi GS, Landoni L, Minetti AE, and Cerretelli P**. Changes in force, cross-sectional area and neural activation during strength training and detraining of the human quadriceps. *Eur J Appl Physiol Occup Physiol* 59: 310-319, 1989.

18. **O'Brien TD, Reeves ND, Baltzopoulos V, Jones DA, and Maganaris CN**. In vivo measurements of muscle specific tension in adults and children. *Exp Physiol* 95: 202-210, 2010.

19. **Pardo JV, Siliciano JD, and Craig SW**. Vinculin is a component of an extensive network of myofibril-sarcolemma attachment regions in cardiac muscle fibers. *J Cell Biol* 97: 1081-1088, 1983.

20. **Quach NL, and Rando TA**. Focal adhesion kinase is essential for costamerogenesis in cultured skeletal muscle cells. *Dev Biol* 293: 38-52, 2006.

21. **Reeves ND, Narici MV, and Maganaris CN**. Effect of resistance training on skeletal muscle-specific force in elderly humans. *J Appl Physiol* 96: 885-892, 2004.

22. **Riechman SE, Balasekaran G, Roth SM, and Ferrell RE**. Association of interleukin-15 protein and interleukin-15 receptor genetic variation with resistance exercise training responses. *J Appl Physiol* 97: 2214-2219, 2004.

23. **Rutherford OM, Greig CA, Sargeant AJ, and Jones DA**. Strength training and power output: transference effects in the human quadriceps muscle. *J Sports Sci* 4: 101-107, 1986.

24. **Rutherford OM, and Jones DA**. The role of learning and coordination in strength training. *Eur J Appl Physiol Occup Physiol* 55: 100-105, 1986.

25. **Rutherford OM, Jones DA, and Newham DJ**. Clinical and experimental application of the percutaneous twitch superimposition technique for the study of human muscle activation. *J Neurol Neurosurg Psychiatry* 49: 1288-1291, 1986.

26. **Seynnes OR, de Boer M, and Narici MV**. Early skeletal muscle hypertrophy and architectural changes in response to high-intensity resistance training. *J Appl Physiol* 102: 368-373, 2007.

27. **Street SF**. Lateral transmission of tension in frog myofibers: a myofibrillar network and transverse cytoskeletal connections are possible transmitters. *J Cell Physiol* 114: 346-364, 1983.

28. **Tabor HK, Risch NJ, and Myers RM**. Candidate-gene approaches for studying complex genetic traits: practical considerations. *Nat Rev Genet* 3: 391-397, 2002.

29. **Tidball JG, Spencer MJ, Wehling M, and Lavergne E**. Nitric-oxide synthase is a mechanical signal transducer that modulates talin and vinculin expression. *J Biol Chem* 274: 33155-33160, 1999.

30. **Tsaopoulos DE, Baltzopoulos V, and Maganaris CN**. Human patellar tendon moment arm length: measurement considerations and clinical implications for joint loading assessment. *Clin Biomech (Bristol, Avon)* 21: 657-667, 2006.

31. **Voight BF, Kudaravalli S, Wen X, and Pritchard JK**. A map of recent positive selection in the human genome. *PLoS Biol* 4: e72, 2006.

32. **Woolstenhulme MT, Conlee RK, Drummond MJ, Stites AW, and Parcell AC**. Temporal response of desmin and dystrophin proteins to progressive resistance exercise in human skeletal muscle. *J Appl Physiol* 100: 1876-1882, 2006.

Tables

Table 1. Baseline values and training-induced changes in muscle strength variables in participants according to protein tyrosine kinase-2 (PTK2) rs7843014 genotype; repeated measures ANOVA *P*-values are presented for genotype (Pre) and training response (Δ) comparisons for the 3 genotypes (P_1), and AA vs. AC + CC (P_2).

	PTK2 rs7843014 genotype					
Strength variable	AA (n = 19)	AC (n = 21)	CC (n = 11)	P_1	AC + CC (n = 32)	P_2
Pre 1-RM (kg)	55.0 ± 13.2	53.8 ± 9.7	54.1 ± 10.9	0.659	53.9 ± 10.0	0.979
Δ 1-RM (%)	64.4 ± 31.9	64.6 ± 28.2	77.0 ± 31.9	0.306	69.0 ± 29.6	0.511
Pre MVC (N·m)	252 ± 58	245 ± 52	245 ± 42	0.826	245 ± 48	0.546
Δ MVC (%)	26.7 ± 8.0	25.4 ± 12.5	26.2 ± 11.9	0.642	25.7 ± 12.1	0.443
Pre SF ($N \cdot cm^{-2}$)	27.7 ± 6.4	24.2 ± 3.7	23.9 ± 4.4	0.078	24.1 ± 3.9*	0.023
Δ SF (%)	16.2 ± 10.5	14.7 ± 11.3	20.0 ± 12.4	0.601	16.5 ± 11.8	0.797

AA homozygote; *AC* heterozygote; *CC* homozygote; *Pre* before training; Δ relative change after training; *1-RM* single repetition maximum; *MVC* maximum isometric voluntary contraction knee joint torque; *SF* quadriceps femoris muscle specific force; *significantly different from AA genotype (post-hoc independent *t*-test: $P = 0.016$).

Table 2. Baseline values and training-induced changes in muscle strength variables in participants according to protein tyrosine kinase-2 (PTK2) rs7460 genotype; repeated measures ANOVA *P*-values are presented for genotype (Pre) and training response (Δ) comparisons for the 3 genotypes (P_1), and TT vs. AT + AA (P_2).

	PTK2 rs7460 genotype					
Strength variable	AA (n = 13)	AT (n = 21)	TT (n = 17)	P_1	AA + AT (n = 34)	P_2
Pre 1-RM (kg)	54.6 ± 9.7	53.0 ± 10.4	55.7 ± 13.4	0.740	53.6 ± 10.0	0.706
Δ 1-RM (%)	69.3 ± 32.3	67.7 ± 27.3	65.2 ± 34.0	0.839	68.4 ± 28.9	0.650
Pre MVC (N·m)	243 ± 47	244 ± 51	256 ± 58	0.697	244 ± 49	0.402
Δ MVC (%)	28.7 ± 11.7	25.1 ± 12.6	25.2 ± 7.0	0.553	26.5 ± 12.2	0.706
Pre SF ($N \cdot cm^{-2}$)	24.0 ± 4.0	24.2 ± 3.6	28.1 ± 6.6	0.058	24.1 ± 3.7**	0.017
Δ SF (%)	20.8 ± 11.9	14.4 ± 11.6	15.5 ± 9.8	0.461	16.9 ± 12.0	0.975

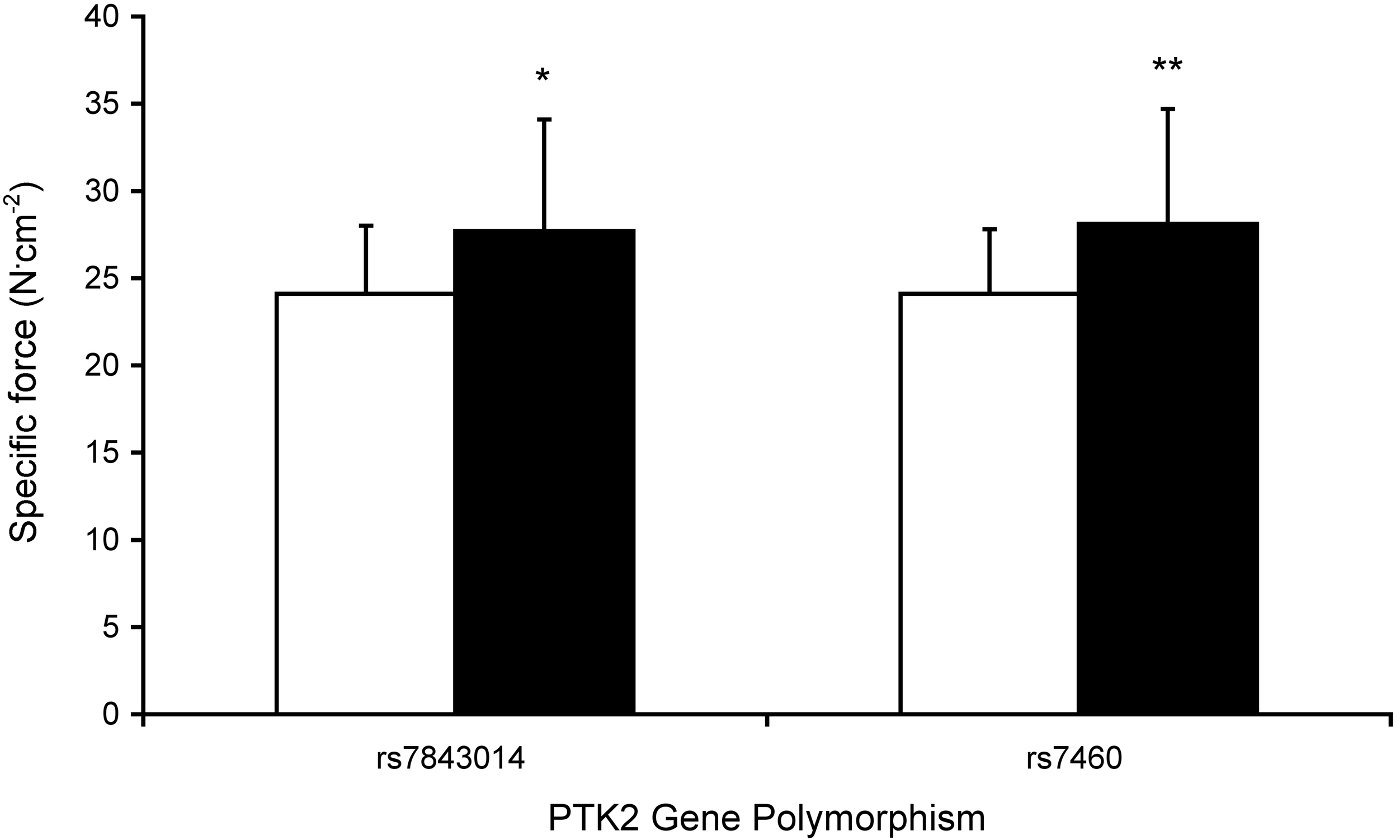
AA homozygote; *AT* heterozygote; *TT* homozygote; *Pre* before training; Δ relative change after training; *1-RM* single repetition maximum; *MVC* maximum isometric voluntary contraction knee joint torque; *SF* quadriceps femoris muscle specific force; **significantly different from TT genotype (post-hoc independent *t*-test: $P = 0.009$).

Table 3. Estimates of haplotype frequencies regarding the protein tyrosine kinase-2 (PTK2) rs7843014 (A/C) and rs7460 (A/T) polymorphisms.

Allele at locus 1 (rs7843014 A/C)	Allele at locus 2 (rs7460 A/T)	Haplotype frequency
A	T	0.519
A	A	0.060
C	T	0.021
C	A	0.401

Figure legend

Fig. 1. Baseline quadriceps femoris muscle specific force according to non-preferential (white bars) and preferential (black bars) genotypes of the protein tyrosine kinase-2 (PTK2) rs7843014 (preferential genotype: AA) and rs7460 (preferential genotype: TT); **P* = 0.016 significantly different from pooled PTK2 rs7843014 AC + CC genotypes; ***P* = 0.009 significantly different from combined PTK2 rs7460 AA + AT genotypes.


40
35
30
25
20
15
10
5
0
Specific force (N·cm-2)
*
**
rs7843014
rs7460
PTK2 Gene Polymorphism