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**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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**Running title:** PTK2 gene variants and strength training

**ABSTRACT**

1 The protein tyrosine kinase-2 (PTK2) gene encodes focal adhesion kinase, a structural  
2 protein involved in lateral transmission of muscle fiber force. We investigated  
3 whether single nucleotide polymorphisms (SNPs) of the PTK2 gene were associated  
4 with various indices of human skeletal muscle strength and the inter-individual  
5 variability in the strength responses to resistance training. We determined unilateral  
6 knee extension single repetition maximum (1-RM), maximum isometric voluntary  
7 contraction (MVC) knee joint torque and quadriceps femoris muscle specific force  
8 (maximum force per muscle physiological cross-sectional area), before and after 9-  
9 weeks of knee extension resistance training in 51 untrained young men. All  
10 participants were genotyped for the PTK2 intronic rs7843014 A/C and 3' UTR rs7460  
11 A/T SNPs. There were no genotype associations with baseline measures or post-  
12 training changes in 1-RM or MVC. Although the training-induced increase in specific  
13 force was similar for all PTK2 genotypes, baseline specific force was higher in PTK2  
14 rs7843014 AA and rs7460 TT homozygotes than in their respective rs7843014 C- ( $P$   
15 = 0.016) and rs7460 A-allele ( $P$  = 0.009) carriers. These associations between muscle  
16 specific force and PTK2 SNPs suggest that inter-individual differences exist in the  
17 way force is transmitted from the muscle fibers to the tendon. Therefore, our results  
18 demonstrate for the first time the impact of genetic variation on the intrinsic strength  
19 of human skeletal muscle.

20

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

23

## 24 INTRODUCTION

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

34

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

46

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

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

54

## 55 **MATERIALS AND METHODS**

### 56 *Participants*

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

69

### 70 *Habitual physical activity rating*

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

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

76

77 *Experimental design*

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

84

85 *Progressive resistance training*

86 The supervised resistance training protocol has been described in detail elsewhere  
87 (10). Briefly, supervised knee extension training was performed unilaterally three  
88 times per week for nine weeks. The maximum training load that could be lifted once  
89 only (1-RM) throughout the full range of knee extension (110° to 20° of knee flexion;  
90 0° = full knee extension) was assessed at the beginning of the training program and  
91 re-evaluated at the start of each week on a standard knee extension machine  
92 (Technogym, Gambettola, Italy). The training intensity was set in relation to the 1-  
93 RM and was therefore progressively increased throughout the nine weeks of training.  
94 Each session comprised a warm-up set of 10 knee extension repetitions at 40% of the  
95 revised 1-RM, followed by four sets (2 min rest between each) of 10 repetitions at  
96 80% 1-RM. Compliance with the training protocol was 100%, with each participant  
97 completing all 27 training sessions.

98

99 *Maximum patellar tendon force*

100 The method used to assess maximum patellar tendon force has been explained in  
101 detail elsewhere (8). In summary, participants performed isometric knee extension  
102 maximal voluntary contractions (MVCs) on a dynamometer (Cybex Norm, Cybex  
103 International, Ronkonkoma, NY) at optimum knee joint angle, which ranged from 70-  
104 90° knee flexion. Participants were seated with a hip angle of 85° (supine = 180°) and  
105 were fixed with inextensible straps to the strength-testing chair. Co-contraction torque  
106 of the antagonist muscles during knee extension MVC was calculated by comparing  
107 electromyographic activity of the biceps femoris muscle during maximal isometric  
108 knee extension and maximal isometric knee flexion (21). Two bipolar silver chloride  
109 surface electrodes (Neuroline, Medicotest, Rugmarken, Denmark) were placed 20 mm  
110 apart along the sagittal axis over the muscle belly (the location was recorded on an  
111 acetate for further tests) and one reference electrode was positioned over the lateral  
112 tibial condyle. The root mean square of the raw EMG signal was calculated over one  
113 second around the peak torque during each maximum voluntary isometric knee  
114 extension and flexion at optimum joint angle and the torque produced by the  
115 hamstrings during knee extension was estimated assuming a linear relationship  
116 between torque and EMG activity (21). The estimated antagonist torque obtained at  
117 the optimum knee extension joint angle was used to calculate the maximum overall  
118 knee extension torque. Voluntary QF muscle activation was assessed using the  
119 interpolated twitch technique (25), whereby the participant received a supramaximal  
120 twitch (Digitimer stimulator model DS7, Welwyn Garden City, UK) via two 7.5 cm x  
121 12.5 cm self-adhesive electrodes (Versastim, Conmed, New York, NY) placed distally  
122 (anode) and proximally (cathode) over the QF muscle, once before MVC (control  
123 twitch) and once during MVC. True maximum torque (TMT) was calculated as:

124 
$$\text{TMT} = \text{MVC}(\text{C}) \cdot (1-t/\text{T})^{-1}$$

125 where  $t$  is the amplitude of the superimposed twitch,  $\text{T}$  is the value of the twitch  
126 before the MVC and  $\text{MVC}(\text{C})$  is MVC corrected for antagonist muscle co-activation.

127 The percentage of voluntary muscle activation was given by:

128 
$$100 \cdot (1-t/\text{T})$$

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

143 
$$F_t = \text{TMT} / d_{\text{PT}}$$

144

145 *Muscle physiological cross-sectional area (PCSA)*

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



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

158

#### 159 *In vivo muscle specific force*

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

164

#### 165 *Blood sampling*

166 A 10-mL blood sample was drawn into 10-mL EDTA tubes (BD Vacutainer Systems,  
167 Plymouth, UK) from a superficial forearm vein. The whole blood was aliquotted into  
168 2-mL tubes (Eppendorf AG, Hamburg, Germany) and stored at  $-80^\circ\text{C}$  until  
169 subsequent analysis.

170

#### 171 *DNA extraction and determination of PTK2 genotype*

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

175

176 Real-time polymerase chain reaction (PCR) was performed to determine the genotype  
177 of the PTK2 polymorphisms in each participant. Reactions were carried out on 96-  
178 well microtiter plates. Each 10- $\mu$ L reaction volume contained: 5- $\mu$ L Genotyping  
179 Master Mix (Applied Biosystems, Foster City, CA), 4.3- $\mu$ L nuclease-free H<sub>2</sub>O  
180 (Qiagen, Crawley, UK), 0.5- $\mu$ L genotyping assay mix (Applied Biosystems, Foster  
181 City, CA), plus 0.2- $\mu$ L sample DNA at a concentration of  $\sim 30$  ng- $\mu$ L<sup>-1</sup> and an  
182 A260/A280 ratio of 1.7–1.9. TaqMan rs7843014 and rs7460 SNP genotyping assay  
183 mixes were used, and each mix included the appropriate TaqMan primers and probes.

184

185 For control wells, 0.2- $\mu$ L nuclease-free H<sub>2</sub>O replaced the DNA template. Following  
186 sealing (Microseal 'B' adhesive seal, BioRad Laboratories, Hercules, CA) and  
187 centrifugation at 8,000 RPM for 1 min, DNA amplification (Chromo4 Real-Time  
188 PCR Detection System, BioRad Laboratories, Hercules, CA) was performed using the  
189 following PCR protocol: denaturation at 95°C for 10 min, followed by 40 cycles of  
190 incubation at 92°C for 15 s then annealing and extension at 60°C for 1 min. PTK2  
191 genotypes were ultimately determined using Opticon Monitor 3.1 software (BioRad  
192 Laboratories, Hercules, CA). All samples were analyzed in duplicate and in all cases  
193 there was 100% agreement between genotype for samples from the same participant.

194

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

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

206

#### 207 *Statistical analysis*

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

220

## 221 **RESULTS**

222 *PTK2 genotypes*

223 The genotype frequencies for the PTK2 rs7843014 (AA = 37.3%; AC = 41.2%; CC =  
224 21.6%) and rs7460 (AA = 25.5%; AT = 41.2%; TT = 33.3%) polymorphisms were all  
225 in Hardy-Weinberg equilibrium ( $P \geq 0.473$ ). Further, the PTK2 rs7843014 A/C and  
226 rs7460 A/T allele frequencies were similar to those reported elsewhere for Caucasian  
227 populations (31).

228

229 *Habitual physical activity rating*

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

235

236 *Single repetition maximum (1-RM)*

237 Baseline 1-RM ( $54.3 \pm 11.0$  kg for the whole cohort) did not differ between genotype  
238 for both the rs7843014 (ANOVA, genotype  $P = 0.659$ ; Table 1) and the rs7460  
239 (ANOVA, genotype  $P = 0.740$ ; Table 1) SNPs. Similarly, the % change in 1-RM  
240 ( $+66.8 \pm 30.2\%$  for the entire group) did not differ between genotype for either SNP  
241 (rs7843014: ANOVA, time x genotype  $P = 0.306$ ; Table 1; rs7460: ANOVA, time x  
242 genotype  $P = 0.839$ ; Table 2).

243

244 *Table 1 near here.*

245

246 *Maximum isometric voluntary contraction (MVC) knee joint torque*

247 Before training, MVC torque ( $248 \pm 52$  N·m for the entire cohort) did not differ  
248 between genotype regarding either the rs7843014 (ANOVA, genotype  $P = 0.826$ ;  
249 Table 1) or the rs7460 (ANOVA, genotype  $P = 0.697$ ; Table 2) SNPs. In addition, the  
250 % change in MVC torque ( $26.1 \pm 10.7\%$  for the whole group) did not differ between  
251 genotype for either SNP (rs7843014: ANOVA, time x genotype  $P = 0.642$ ; Table 1;  
252 rs7460: ANOVA, time x genotype  $P = 0.553$ ; Table 2).

253

254 *Table 2 near here.*

255

256 *Muscle physiological cross-sectional area (PCSA)*

257 Prior to training, QF muscle PCSA for the total cohort was  $239 \pm 40$  cm<sup>2</sup>, and there  
258 was no association with either SNP (ANOVA, genotype  $P \geq 0.314$ ). Nine weeks of  
259 resistance training led to a  $5.8 \pm 4.5\%$  increase in muscle PCSA (ANOVA, time  $P <$   
260  $0.0005$ ), which was independent of PTK2 genotype (ANOVA, time x genotype  $P \geq$   
261  $0.963$ ).

262

263 *Muscle specific force*

264 Regarding untrained muscle specific force ( $25.5 \pm 5.2$  N·cm<sup>-1</sup> for the entire group),  
265 there were non-significant tendencies for PTK2 rs7843014 AA homozygotes to  
266 produce higher muscle specific force than their AC and CC counterparts (ANOVA  
267 genotype  $P = 0.078$ ; Table 1), and the muscles of PTK2 rs7460 TT homozygotes to  
268 have higher specific force than AA and AT genotypes (ANOVA, genotype  $P = 0.058$ ;  
269 Table 2). When the PTK2 rs7843014 AC and CC genotypes were pooled, the QF  
270 muscles of individuals homozygous for the A-allele expressed higher specific force  
271 than carriers of the C-allele before training (ANOVA, genotype  $P = 0.023$ ; Table 1; *t-*

272 test  $P = 0.016$ ; Fig. 1). Similarly, when the PTK2 rs7460 AA and AT genotypes were  
273 combined, QF muscle specific force was found to be higher in TT homozygotes than  
274 in A-allele carriers before training (ANOVA, genotype  $P = 0.017$ ; Table 2;  $t$ -test  $P =$   
275  $0.009$ ; Fig. 1). However, there was no significant interaction between training and  
276 PTK2 genotype concerning QF muscle specific force and both the rs7843014  
277 (ANOVA, time x genotype  $P = 0.601$ ; time  $P < 0.0005$ ; Table 1) and rs7460  
278 (ANOVA, time x genotype  $P = 0.461$ ; time  $P < 0.0005$ ; Table 2) PTK2 SNPs,  
279 implying that specific force increased similarly among all three genotypes of both  
280 SNPs ( $16.4 \pm 11.2\%$  for the whole cohort).

281

282 *Fig. 1 near here*

283

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

292

293 *Table 3 near here.*

294

295 Both PTK2 SNPs were associated with untrained muscle specific force, therefore the  
296 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

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

323

324 The genotype frequencies for the PTK2 rs7843014 (AA = 37%; AC = 41%; CC =  
325 22%) and rs7460 (AA = 26%; AT = 41%; TT = 33%) SNPs observed in our study  
326 were comparable to those reported previously for Caucasian populations (31).

327 Baseline values for our entire cohort were similar to those reported elsewhere for this  
328 population concerning 1-RM lifting strength (13), isometric MVC knee joint torque  
329 (18), QF muscle PCSA (16) and specific force (16). Our observed 67% increase in 1-  
330 RM for the whole cohort was higher than some (22), but less than other (23, 24)  
331 reports of 1-RM strength gains following a similar period of knee extensor strength  
332 training. The 26% increase in isometric knee extensor MVC strength was less than  
333 some (26), but greater than other (1, 17) previously reported gains in isometric  
334 strength following a similar duration of knee extensor training. Regarding muscle  
335 hypertrophy, our observed 6% increase in QF muscle PCSA was comparable to  
336 previous reports of QF muscle size gains following resistance training of similar type  
337 and duration (1, 17). The 16% increase in muscle specific force was also comparable  
338 to that reported elsewhere following resistance training of the QF muscle, although in  
339 older individuals (21).

340

341 Focal adhesion kinase (FAK) plays an integral role in the costamere protein complex  
342 (4, 20) that is involved in the lateral transmission of force (6). As FAK is encoded by  
343 the PTK2 gene, we hypothesized that polymorphisms of this gene would explain part  
344 of the inter-individual variability in QF muscle specific force between untrained  
345 young men. We determined that individuals homozygous for the rs7843014 A-allele



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

349

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

368

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

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

378

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

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

405

#### 406 *Summary and conclusions*

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

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## 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 ( $\Delta$ ) comparisons for the 3 genotypes ( $P_1$ ), and AA vs. AC + CC ( $P_2$ ).

Strength variable	PTK2 rs7843014 genotype			$P_1$	AC + CC ( $n = 32$ )	$P_2$
	AA ( $n = 19$ )	AC ( $n = 21$ )	CC ( $n = 11$ )			
Pre 1-RM (kg)	55.0 ± 13.2	53.8 ± 9.7	54.1 ± 10.9	0.659	53.9 ± 10.0	0.979
$\Delta$ 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
$\Delta$ MVC (%)	26.7 ± 8.0	25.4 ± 12.5	26.2 ± 11.9	0.642	25.7 ± 12.1	0.443
Pre SF (N·cm <sup>-2</sup> )	27.7 ± 6.4	24.2 ± 3.7	23.9 ± 4.4	0.078	24.1 ± 3.9*	0.023
$\Delta$ 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;  $\Delta$  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 ( $\Delta$ ) comparisons for the 3 genotypes ( $P_1$ ), and TT vs. AT + AA ( $P_2$ ).

Strength variable	PTK2 rs7460 genotype			$P_1$	AA + AT ( <i>n</i> = 34)	$P_2$
	AA ( <i>n</i> = 13)	AT ( <i>n</i> = 21)	TT ( <i>n</i> = 17)			
Pre 1-RM (kg)	54.6 ± 9.7	53.0 ± 10.4	55.7 ± 13.4	0.740	53.6 ± 10.0	0.706
$\Delta$ 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
$\Delta$ MVC (%)	28.7 ± 11.7	25.1 ± 12.6	25.2 ± 7.0	0.553	26.5 ± 12.2	0.706
Pre SF (N·cm <sup>-2</sup> )	24.0 ± 4.0	24.2 ± 3.6	28.1 ± 6.6	0.058	24.1 ± 3.7**	0.017
$\Delta$ 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;  $\Delta$  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.

