Is *PPARα* intron 7 G/C polymorphism associated with muscle strength characteristics in nonathletic young men?

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Peroxisome proliferator-activated receptor alpha (*PPARα*), a ligand-dependent transcription factor, regulates fatty acid metabolism in heart and skeletal muscle. The intron 7 G/C polymorphism (rs4253778) has been associated with athletic performance. The rare C-allele was predominant in power athletes, whereas the G-allele was more frequent in endurance athletes. In the present study, we investigated the association between this polymorphism and strength characteristics in nonathletic, healthy young adults ($n = 500$; age 24.2 ± 4.4 years). Knee torque was measured during concentric knee flexion and extension movements at 60°/s, 120°/s, and 240°/s during 3, 25, and 5 repetitions,

respectively. Also, resistance to muscle fatigue (i.e. work last 20% repetitions/work first 20% repetitions $\times 100$) was calculated. Differences in knee strength phenotypes between GG homozygous individuals and C-allele carriers were analyzed. The polymorphism did not influence the ability to produce isometric or dynamic knee flexor or extensor peak torque during static or dynamic conditions in this population ($0.23 < P < 0.95$). Similar results were found for the endurance ratio, a measure for resistance to muscle fatigue. In conclusion, the *PPARα* intron 7 G/C polymorphism does not seem to influence strength characteristics in a nonathletic population.

The peroxisome proliferator-activated receptors (*PPARs*) are members of the nuclear hormone receptor superfamily involved in lipid metabolism and glucose homeostasis. Three different subtypes have been identified: *PPARα*, *PPARβ/δ*, and *PPARγ*, each encoded by a different gene. Mechanistically, all *PPARs* form heterodimers with the nuclear retinoid X receptor and bind to specific peroxisome proliferator hormone response elements within the promoters of the target genes (Kliwer et al., 1992). The receptors function by altering gene transcription when activated by a ligand after recruitment of coactivators or corepressors (Tien et al., 2006).

PPARα regulates expression of genes involved in multiple steps of the lipid metabolism, including fatty acid transport, uptake, and oxidation (Gulick et al., 1994; Schmitt et al., 2003). It is therefore expressed at high levels in tissues that mainly depend on fatty acid oxidation, such as brown adipose tissue, liver, heart, and skeletal muscle (Desvergne & Wahli, 1999; Bookout et al., 2006).

In humans, the expression of *PPARα* is higher in type I than in type II muscle fibers (Russell et al., 2003;

Schmitt et al., 2003). This is in line with earlier findings that type I fibers depend highly on fatty acid oxidation, while type II fibers mainly use glycolysis for energy production. *PPARα* increases skeletal fatty acid oxidation capacity by enhancing the expression of important proteins of the lipid metabolism, such as fat carriers *FATP*, *FAT/CD36*, and *CPT-1* (Mascaro et al., 1998; Motojima et al., 1998). The muscle *PPARα* content can be increased by endurance training, with the highest increase in type I fibers (Horowitz et al., 2000; Russell et al., 2003).

Endurance exercise improves fatty acid oxidation capacity by increasing the expression of proteins involved in lipid metabolism (Cresci et al., 1996), as fatty acids are the primary source of energy during endurance exercise. It is therefore suggested that a higher *PPARα* protein content is beneficial for endurance-type exercise. This hypothesis is strengthened by the observation of elevated skeletal muscle mRNA expression of *PPARα* in endurance cyclists compared to moderately active men (Kramer et al., 2006).

Since genetic variation can influence gene expression and protein content, polymorphisms in the *PPAR α* gene could alter the level of the *PPAR α* protein in the muscle cells and as a result affect fatty acid oxidation. In humans, several single-nucleotide polymorphisms (SNPs) in the *PPAR α* gene (chromosome 22) have been reported. Variation in the gene has been associated with coronary artery disease and with alterations in lipid and cholesterol concentrations (L162V, rs1800206; Flavell et al., 2002). Recently, a Val227Ala substitution in the *PPAR α* gene has been shown to result in a decreased waist-to-hip ratio and waist circumference and in an elevated risk for nonalcoholic liver disease (Chen et al., 2008). In the context of genetic variation in the *PPAR α* gene and exercise/performance-related research, the most frequently SNP studied is the intron 7 G/C polymorphism (rs4253778). The exact function of this SNP is speculative. Regulatory regions have been found in introns; however, it is unlikely that the intron 7 variant is itself functional as it is not close to the splice branch point sequence (Foucher et al., 2004). However, clear associations with clinical phenotypes have been reported for the intron 7 G/C SNP variant. The C-allele was found to be associated with an elevated risk for type 2 diabetes (Andrulionyte et al., 2007) and cardiac hypertrophy (Jamshidi et al., 2002). The increase in cardiac muscle mass has been associated with a decrease in fatty acid oxidation and in *PPAR α* protein content (Allard et al., 1994; Barger et al., 2000) that leads to the hypothesis that the C-allele lowers the amount of *PPAR α* in the muscle.

A difference in *PPAR α* protein content and fatty acid oxidation between intron 7 G/C genotype groups could also have an influence on physical performance. A decrease in fatty acid oxidation, as is hypothesized in intron 7 C-allele carriers, could be a disadvantage during endurance-type activities. Evidence was provided by Ahmetov et al. (2006) who showed that the C-allele is more common in Russian power athletes, whereas the frequency of the G-allele is higher in endurance athletes. Similar results were found in Israeli (Eynon et al., 2010) and Polish athletes (Maciejewska et al., 2011). In contrast, event time at the Olympus Marathon in Greek endurance athletes was not associated with the polymorphism (Tsianos et al., 2010). In addition, GG homozygotes showed higher percentages of oxidative type I muscle fibers compared to CC homozygotes (Ahmetov et al., 2006).

On the other hand, the decreased proportion of type I fibers in CC homozygotes may be beneficial during power-oriented sports. Also, it is suspected that the reduction of *PPAR α* in the muscle is one of the key factors in activating the anaerobic pathways of glucose utilization (Barger et al., 2000). Thus, variation in the *PPAR α* gene could influence glucose production and handling in the muscle, which may be expressed during power tests. In line, Ahmetov et al. (2006) found an

increasing linear trend of the C-allele with increasing anaerobic component of physical performance. These data suggest that the G-allele is associated with increased endurance capacity, whereas the C-allele could be an advantage in power-type activities.

Until now, studies of the *PPAR α* intron 7 G/C polymorphism have been limited to case-control studies in athletes and the polymorphism has not yet been related to muscle strength characteristics in nonathletic individuals. This study will therefore examine the influence of the polymorphism on knee torque delivered during maximal and dynamic flexor and extensor movements in a normal, healthy population. Based on previous data (Ahmetov et al., 2006; Maciejewska et al., 2011), it is hypothesized that isometric and dynamic torque are higher in CC individuals.

In addition, the endurance ratio is included as a measure of resistance to muscle fatigue. Avoiding or delaying the decline in the capacity to generate force over repeated contractions in time can result in an improved performance. When compared to power athletes, endurance athletes showed better results on fatigue parameters assessed in isokinetic knee flexion and extension fatiguing protocols similar to our muscle fatigue endurance test (Rainoldi et al., 2008). Hence, we hypothesize that the G-allele is associated with elevated resistance to muscle fatigue of the knee flexor and extensor muscles.

Materials and methods

Subjects

Five hundred male siblings of 235 Caucasian families aged 24.2 years [standard deviation (SD) = 4.4] were selected as a subsample from the 748 participants in the Leuven Genes for Muscular Strength study. All volunteered to participate in this study and written informed consent was obtained from each individual. The study protocol was approved by the medical and ethical committee of the Faculty of Medicine of the Katholieke Universiteit Leuven.

Measurements

A detailed overview of the anthropometrical and muscle strength measurements is provided by Huygens et al. (2004, 2005). The phenotypes, selected as dependent variables in this specific study, are described below.

Body composition

Anthropometric measurements were taken from all subjects to calculate fat-free mass and percentage body fat. Skinfolds of the triceps, biceps, subscapular, and suprailiacal region were measured to the nearest 0.1 mm using a Harpenden skinfold calliper (Baty, British Indicators, Burgess Hill, West Sussex, UK) to estimate fat-free mass using the standard Durnin and Womersley equation (Durnin & Womersley 1974): $D = 1.1765 - 0.0744 \times \log_{10}(\text{sum of skinfolds})$. The Siri equation was used to assess percentage body fat (Siri, 1993): $\text{Body fat (\%)} = (4.95/D - 4.50) \times 100$. Cross-sectional muscle and bone area (MBA) of the thigh (cm^2) was estimated based on the circumference of the mid-thigh corrected for subcutaneous fat at this

location: $MBA = [\text{circumference mid-thigh} - (\text{skinfold thickness mid-thigh} \times \pi/10)^2]/(4 \times \pi)$. All measurements were taken by experienced anthropometrists in standardized conditions.

Strength measurements

All subjects reported to the research center for several strength tests. After a warm-up session of 5–10 min on a cycle ergometer and stretching exercises, isometric and concentric torques of the knee muscles were measured using a Cybex NORM isokinetic dynamometer (Lumex Inc., Ronkonkoma, New York, USA). The subject was positioned into the test seat according to the guidelines of the manufacturer. The mechanical axis of the Cybex was aligned with the rotation axis of the knee joint. Anatomical zero was preset at full extension of the limb. After a series of familiarization contractions, isometric strength was measured. According to the force–length relationship of a muscle, optimal strength is generated at longer muscle length. This corresponds to an angle of 30° for knee flexion (hamstrings) and 60° for knee extension (quadriceps). At these angles, the highest torque values (Nm) during a 6-s isometric contraction of three maximal flexion and extension contractions were retained for further analysis. The subjects were given 30 s of rest after each contraction. Knee torque of concentric knee extension and flexion were determined during contractions over the complete range of motion (0–90°) at a velocity of 60°/s, 240°/s, and 120°/s during 3, 5, and 25 repetitions, respectively.

The endurance ratio was calculated as a measure of resistance to muscle fatigue during the 25 repetitions by dividing the work in the last 20% of repetitions by the work in the first 20% of repetitions of the set, multiplied by 100 (Fig. 1). Subjects were verbally encouraged to perform at their maximum effort and visual feedback of their performance was presented after each test.

DNA collection and genotyping

DNA was extracted from ethylenediaminetetraacetic acid whole blood via a standard salting out method (Miller et al., 1988) or from saliva collected in an Oragene DNA self-collection kit (Oragene, Kanata, Ontario, Canada) according to the protocol provided by the manufacturer.

The intron 7 G/C polymorphism (rs4253778) was genotyped using an Illumina BeadArray platform (Illumina Inc., San Diego, CA, USA). Genotypes were determined with GenCall 5.2.0 software (Illumina Inc., San Diego, CA, USA.) and visually checked.

Fig. 1. Endurance ratio is calculated as the ratio of work done during the last 20% of the set and work done during the first 20% of the set, multiplied by 100. Endurance ratio = (work last 20% reps/work first 20% reps) $\times$ 100. Adapted from Thomis et al. (2011).

Statistical analysis

A chi-square goodness-of-fit test (degree of freedom = 1) was performed to determine deviations of genotype distribution from Hardy–Weinberg equilibrium. Anthropometric and strength measurements were compared between C-allele carriers (CG+CC individuals) and GG homozygotes using Student *t*-tests and analysis of covariance (ANCOVA) using a proc mixed procedure with a “sandwich” option to account for dependency between scores within families in SAS version 9.2 (SAS Institute, Cary, North Carolina, USA). Power calculations were performed based on estimated effect sizes and observed sample sizes using proc power. For the present sample sizes, an effect size of 0.26 can be detected with 80% power. This corresponds to about 2.3% of explained variance, thus a relatively small genetic effect.

Results

Prevalence of the 7 G/C polymorphism

In this study, the proportion of GG, GC, and CC genotypes was 64.4%, 32.2%, and 3.4%, respectively. This corresponds to an allele frequency of 0.80 for the G-allele and 0.20 for the C-allele. The observed frequencies of the 7 G/C polymorphism are consistent with reports of general Russian (Ahmetov et al., 2006), Israeli (Eynon et al., 2010), and Polish populations (Cieszczyk et al., 2011; Maciejewska et al., 2011). The genotype distribution was in agreement with Hardy–Weinberg equilibrium ($\chi^2 = 0.33$; $P = 0.56$).

Body composition

Somatic characteristics and estimated muscle bone cross-sectional area of the thigh were similar across the different genotype groups (Table 1) apart from % body fat that was lower in GG homozygotes compared to C-allele carriers ($P = 0.045$).

Strength characteristics

As shown in Fig. 2, isometric torque production was similar between GG homozygotes and C-allele carriers during flexion ($P = 0.67$) and extension ($P = 0.72$) movements. According to the torque-velocity relationship, torque production decreased as contraction velocity

Table 1. Anthropometric characteristics according to intron 7 G/C genotype

	G/G	C/C + G/C	<i>P</i> value*
<i>n</i>	322	178†	
Age, years	23.5 $\pm$ 0.6	23.6 $\pm$ 0.6	0.66
Stature, cm	180.1 $\pm$ 0.5	180.4 $\pm$ 0.6	0.52
Weight, kg	72.3 $\pm$ 0.9	73.8 $\pm$ 1.1	0.078
Body fat, %	14.7 $\pm$ 0.5	15.8 $\pm$ 0.6	0.045
Fat-free mass, kg	61.3 $\pm$ 0.6	61.8 $\pm$ 0.7	0.41
Muscle bone area, cm ²	170.1 $\pm$ 2.1	169.6 $\pm$ 2.3	0.81

Values are LSmeans $\pm$ SE.

**t*-test comparing both genotype groups.

†Genotyping failed for one individual.

Fig. 2. Effect of *PPAR* α intron 7 G/C polymorphism on knee flexion and extension torque-velocity curve. Torques were measured at 30° for knee flexors and at 60° for extensors. Error bars are SEM.

Table 2. The endurance ratio according to intron 7 G/C genotype

	G/G	C/C + G/C	<i>P</i> value*
Flexion, %	75.6 $\pm$ 0.8	76.8 $\pm$ 0.9	0.21
Extension, %	77.7 $\pm$ 0.7	79.0 $\pm$ 0.8	0.09

Values are LSmeans $\pm$ SE.

*ANCOVA comparing both genotype groups including age, weight, and % body fat as covariates.

increased (test for main effect of velocity: $P < 0.0001$). Allele-dependent differences in torques at each contraction velocity were nonsignificant ($0.23 < P < 0.95$). Also, the allele by velocity interaction showed no significant differences for flexors ($P = 0.91$) and extensors ($P = 0.50$), indicating that the torque-velocity curves follow the same pattern, independent of *PPAR* α alleles. These results were unaffected by inclusion or exclusion of covariates age, weight, and percentage body fat.

A comparison between GG homozygotes and C-allele carriers for the endurance ratio can be found in Table 2. No significant differences were found.

Discussion

The present study is the first report regarding the *PPAR* α intron 7 G/C polymorphism and strength-related characteristics in nonathletic, healthy young adults. Our results indicate that the polymorphism does not influence the ability to produce isometric or dynamic peak torques in nonathletic individuals when performed in a controlled setting. Although the athlete control association study of Ahmetov et al. (2006) showed an increased frequency of the C-allele in power-oriented athletes, our data do not support a beneficial role for the *PPAR* α intron 7 C-allele for moderately high velocity contractions or for maximal isometric strength.

Athletic performance such as sprinting requests the generation of power in repeated cycles of muscle contractions at high angular velocities. This is in contrast to the strength phenotypes in the present study, which were assessed based on a limited number of muscle contractions at relatively low angular velocities (60°/s, 120°/s, and 240°/s). Sprinting also requires whole body movement, whereas muscle strength characteristics of the knee extensors and flexors were evaluated with isolated isokinetic tests based on single-joint movements.

In addition, human physical performance is a complex, multifactorial phenotype influenced by many genes and environmental factors, but also by gene–gene and gene–environment interactions (Bray et al., 2009). The failure of this study to confirm the previous association findings could be caused by these interactions. There may be (unknown) polymorphisms in this gene that predominate the real effect of the investigated polymorphism. Previous studies have stated that the intron 7 C-allele is in allelic association with the V162 allele, which encodes a more transcriptionally active *PPAR* α and attenuates the effect of the intron 7 C-allele, e.g. on exercise-induced left ventricular hypertrophy (Jamshidi et al., 2002). On top, it is suggested that the C-allele of the *PPAR* α intron 7 G/C polymorphism is in linkage disequilibrium with a functional variant in a promoter or enhancer element of the *PPAR* α gene that results in reduced *PPAR* α gene expression. In view of the polymorphic site location, the variant may disrupt a microRNA site or be in linkage disequilibrium with a SNP that disrupts a microRNA site (Cresci et al., 2008). As a result of chronic exercise training, endurance athletes have an elevated mRNA expression of *PPAR* α when compared with normally active subjects (Kramer et al., 2006). After an endurance program of 6 weeks, the *PPAR* α mRNA expression increased by 3.0-fold and 1.5-fold in type I and type II skeletal muscle fibers, respectively. The increased *PPAR* α mRNA levels have been associated with an augmented percentage of type I skeletal muscle fibers in humans (Kramer et al., 2006). Combined with the results of Ahmetov et al. (2006) who found an increased percentage of type I skeletal muscle fibers in GG individuals compared to CC homozygotes, these data deliver indirect evidence that G-allele carriers have higher *PPAR* α mRNA levels than individuals carrying the C-allele. As seen in many other genes, the transcriptional adaptation may result into an altered *PPAR* α protein content in skeletal muscle cells.

PPAR α plays an important role in fat metabolism. Lower protein content could therefore lead to a reduced fatty acid oxidation rate in skeletal muscles, a disadvantage for endurance-type activities. In *PPAR* α -null mice, a decrease of 28% in fatty acid metabolism was induced when the mice were starved for 24 h (Muoio et al., 2002a). On the other hand, overexpressing *PPAR* α in skeletal muscle of mice resulted in an upregulation of several target genes of *PPAR* α involved in fatty

acid oxidation, whereas glucose utilization and uptake decreased (Finck et al., 2005).

The decreased *PPARα* content may be (partially) mediated by *PPARβ/δ*. This *PPAR* isoform is the most abundantly expressed *PPAR* in skeletal muscle cells. The expression of *PPARβ/δ* is also higher in type I skeletal muscle fibers than in type II fibers as is observed for *PPARα* (Ehrenborg & Krook, 2009). Moreover, *PPARα* and *PPARβ/δ* seem to overlap in function. Both are able to activate genes involved in various regulatory steps of the lipid metabolism in skeletal muscle such as fatty acid oxidation and transport (Muio et al., 2002b; Tanaka et al., 2003; Kramer et al., 2007). Thus, it may be possible that *PPARβ/δ* fills in the shared functions in the absence of *PPARα*. Indirect evidence was provided by Muio et al. (2002a) who found no difference in skeletal muscle fatty acid oxidation rate between *PPARα*-null mice and wild type mice under baseline conditions. More important, when activated, the increases in mRNA expression of genes involved in the fat metabolism mediated by *PPARβ/δ* were similar to that of *PPARα*. Thus, it is possible that in the absence of *PPARα*, the *β/δ* isotype, in part or completely, takes over its functions.

Remarkably, fatty acid oxidation rate was unchanged in *PPARα*-null mice when fed, whereas the rate dropped significantly when the mice were starved for 24 h (Muio et al., 2002a). As the effect of starvation on the fat metabolism might in many aspects be similar to the alterations during exhaustive endurance exercises, this may explain why an association was found between the *PPARα* intron 7 G/C polymorphism and athletic status while no effect was observed with muscle characteristics in the general population used in this study. Under normal circumstances, *PPARβ/δ* may be able to compensate for the lower *PPARα* content inherent to CC individuals, whereas this may not be possible during extreme conditions such as intensive training. Therefore, the decreased *PPARα* content, caused by the C-allele, could explain the low frequency of the CC genotype in endurance athletes.

In accordance, Maciejewska et al. (2011) found the G-allele to be more frequently present in Polish rowers compared to sedentary controls. Moreover, GG homozygotes showed significantly higher percentages of type I fibers (Ahmetov et al., 2006). These slow-twitch fibers have a high resistance to fatigue compared to type II fibers what resulted in the hypothesis that the endurance ratio would be associated with the G-allele. However, no significant differences in endurance ratio were found between GG homozygotes and C-allele carriers (GC+CC) in this nonathletic population, neither for flexion ($P = 0.21$) nor for extension ($P = 0.09$). Likewise, there was no evidence for linkage for overall maximal endurance capacity as well as resistance to muscle fatigue phenotypes at chromosome 22 (Bouchard et al., 2000; Thomis et al., 2011).

We found the percentage body fat to be higher in C-allele carriers compared to individuals with the GG genotype. In accordance, elevated lipid levels have been associated with the C-allele in type 2 diabetic patients (Doney et al., 2005). To our knowledge, no study has reported this result in a healthy population. Nevertheless, the difference in body fat (%) between the two groups did not affect our results as the P values were similar when percentage body fat was included or excluded as a covariate in an ANCOVA analysis.

To reduce the risk of error due to the small number of CC homozygotes in this study sample ($n = 17$), a carrier analysis was performed. As an elaboration, the same analyses were performed using the three genotype groups separately (data not shown). No differences were found between genotype groups in anthropometric measurements. All muscle strength characteristics were similar among the three genotype groups, except for the endurance ratio of the knee flexors. Post-hoc analysis showed that CC homozygotes had significant higher resistance to muscle fatigue index compared to GC and GG genotype groups ($P = 0.034$). This result seems contrary to the stated hypothesis. However, the test only includes 25 all-effort repetitions, which represents a power decline test of short duration. Delaying the decline in force generation over repeated contractions can prove to be an advantage in sports that require maximal, short duration movements as typical for various power-oriented activities. Nevertheless, keeping the low frequency of CC homozygotes in mind, these results have to be interpreted with caution.

In summary, although our study was sufficiently powered to detect allelic effects of 0.26 SD or 2–3% explained variance, the results indicate that the *PPARα* intron 7 G/C polymorphism has little or no effect on strength-related phenotypes in nonathletic young men. Isometric and concentric peak torque was found to be similar between GG homozygotes and C-allele carriers (GC+CC). Also, no differences were found in muscular endurance ratio between the two groups. The impact of this individual polymorphism seems to be of insufficient magnitude to influence muscle characteristics in a non-athletic population.

Perspectives

The *PPARα* intron 7 G/C polymorphism was presumed to be a genetic factor that could contribute to the orientation or reorientation of sport discipline based on the alleles of the athlete. However, our data show no effect on muscle characteristics in young adults, reducing the possibility to predispose individuals to a certain discipline based on this polymorphism. As this is the first rapport on the association between the 7 G/C polymorphism and strength characteristics in a nonathletic population, further studies are needed to

unravel the true effect of the PPAR α intron 7 G/C polymorphism.

Key words: genotype-phenotype association, genetic variation, SNP, PPAR alpha.

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