CNTF 1357 G → A polymorphism and the muscle strength response to resistance training

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Submitted 30 June 2008; accepted in final form 6 July 2009

Walsh S, Kelsey BK, Angelopoulos TJ, Clarkson PM, Gordon PM, Moyna NM, Visich PS, Zoeller RF, Seip RL, Bilbee S, Thompson PD, Hoffman EP, Price TB, Devaney JM, Pescatello LS. CNTF 1357 G → A polymorphism and the muscle strength response to resistance training. J Appl Physiol 107: 1235–1240, 2009. First published July 23, 2009; doi:10.1152/japplphysiol.90835.2008.—The present study examined associations between the ciliary neurotrophic factor (CNTF) 1357 G → A polymorphism and the muscle strength response to a unilateral, upper arm resistance-training (RT) program among healthy, young adults. Subjects were 754 Caucasian men (40%) and women (60%) who were genotyped and performed a training program of the nondominant (trained) arm with the dominant (untrained) arm as a comparison. Peak elbow flexor strength was measured with one repetition maximum, isometric strength with maximum voluntary contraction, and bicep cross-sectional area with MRI in the trained and untrained arms before and after training. Subjects with the CNTF GG genotype gained more absolute isometric strength, as measured by 1 RM, than carriers of the CNTF A1357 allele in the trained arm pre- to posttraining (P < 0.05). No significant associations were seen in men. Subjects with the CNTF GG genotype gained more absolute dynamic (1.0 ± 0.1 vs. 0.6 ± 0.1 kg) and allometric (0.022 ± 0.0 vs. 0.015 ± 0.0 kg/kg·0.67 kg·0.67) strength, as measured by 1 RM, than carriers of the CNTF A1357 allele in the untrained arm pre- to posttraining (P < 0.05). No significant associations were seen in men. No significant associations, as measured by cross-sectional area, were seen in men or women. The CNTF 1357 G → A polymorphism explains only a small portion of the variability in the muscle strength response to training in women.

CILIARY NEUROTROPIC FACTOR (CNTF) is a member of the IL-6 family of cytokines. CNTF is a pleiotropic signaling molecule that acts as a chemical communicator by binding to its receptor, CNTFR, in target tissues, such as motor neurons and skeletal muscle (9). CNTF has neurotrophic and myotrophic roles (5) and supports survival and differentiation in a variety of neuronal cell types, including motor neurons (19). Local exogenous administration of CNTF in the soleus muscle of rats increased muscle cross-sectional area (CSA) and strength (6).

Takashi et al. (20) first reported a G-A substitution (1357 G → A) in the second exon of the gene resulting in a detectable level of a functional CNTF protein in individuals homozygous for the A1357 allele (A/A). Roth and colleagues (17) examined the role of this CNTF genetic variant as it relates to muscle strength in 494 men and women aged 20–90 yr. Subjects heterozygous for the null mutation of the CNTF 1357 G → A single-nucleotide polymorphism (SNP) exhibited significantly greater concentric peak torque (180°/s angular velocity) in knee extensors and flexors than individuals with the G1357A genotype. Using Biodex dynamometry, De Mars et al. (3) investigated genetic variation in the CNTF and CNTFR genes and their relationships to muscle strength of the knee extensors and flexors in 493 men and women aged 38–80 yr. They found an association between CNTF 1357 G → A polymorphism and strength in men and showed inconclusive results for a limited number of strength phenotypes in women (3). Arking et al. (1) genotyped eight CNTF SNPs, including the CNTF 1357 G → A polymorphism, and examined their respective associations with bilateral grip strength in 363 women aged 70–79 yr. A haplotype analysis was performed. A single haplotype that associated grip strength with the CNTF 1357 G → A polymorphism emerged, fully explaining the association between this haplotype and grip strength. Women homozygous for the null allele (A1357A) exhibited significantly lower nondominant grip strength than those carrying the G1357A allele (1). Thus the research that has been conducted on CNTF gene variation and muscle strength phenotypes is limited, has been primarily cross-sectional, and has yielded mixed results.

No studies have examined the influence of the CNTF 1357 G → A SNP on the muscle strength response to a 12-wk unilateral, upper arm resistance-training program among a large sample of healthy, young adults. Therefore, the purpose of the present study was to examine whether the CNTF 1357 G → A polymorphism influences the muscle strength and size response to resistance training among college-aged, healthy Caucasian men and women. On the basis of the limited work
that suggests that the CNTF A1357 allele is associated with loss of functional CNTF protein (20) and lower muscle strength (1), we hypothesized that individuals carrying the A1357 allele would exhibit significantly lower gains in muscle strength after completion of a 12-wk resistance-training program than those homozygous for the CNTF G1357 allele.

Methods

This study evolved from a larger project, “Functional Single Nucleotide Polymorphisms Associated with Human Muscle Size and Strength” (FAMuSS), conducted by the Exercise and Genetics Collaborative Research Group. Details of the FAMuSS recruitment and methodology are described elsewhere (2). The study protocol was approved by the institutional review boards from the 10 sites involved with FAMuSS, and informed consent was obtained from the subjects.

Subjects. Study participants were healthy men and women, aged 18–39 yr. Individuals did not qualify for participation if they self-reported a history of resistance training during the prior year, use of protein supplements during the prior 3 mo, or alcohol consumption >14 drinks/wk. Those who disclosed a medical history of heart conditions, diabetes mellitus, claustrophobia, or pregnancy were excluded. In addition, those reporting use of antihypertensives, antilipemics, diuretics, clembuterol, anabolic steroids, prednisone, nonsteroidal anti-inflammatories, Depo-Provera, hydrocortisone, or lithium were excluded. Furthermore, individuals with metallic implants were excluded from study participation.

Anthropometric measurements. Body weight and height were measured and recorded before and after training. After the subjects removed their shoes and heavy clothing, body weight was determined using a standard balance beam scale (model 338 Eye-Level Physician Scale, Detectoscale, Webb City, MO). Body height was recorded in inches. Body mass index (kg/m²) was then calculated.

One-repetition maximum strength testing. Dynamic elbow flexor muscle strength was assessed bilaterally using one repetition maximum (1 RM). Testing occurred before training and 48 h after the final training session. Subjects were seated on a standard preacher curl bench (Yukon International, Cleveland, OH), such that a 90° angle was formed at the knee joints, and the chest pad was positioned 2–3 inches below the axilla. With the arm in a fully flexed position, the subject was given a Powerblock (Intellbell, Owatonna, MN) weighing 40–60% of estimated 1 RM and performed 5–10 full range-of-motion repetitions. After a 1-min rest period, the weight was increased to 60–80% of 1 RM, and the subject performed three to five repetitions with this weight. After a 3-min rest period, the weight was increased to predicted 1 RM, and the subject performed one repetition of this weight. If the lift was unsuccessful, the weight was reduced by 2.5 lb, the subject rested for 3 min, and the lift was retried. If the lift was successful, the weight was increased by 2.5 lb, the subject rested for 3 min, and the lift was attempted again. This process was repeated until the subject failed to complete one full range-of-motion repetition. Maximum weight (kg) lifted was recorded as the greatest weight successfully lifted one time. Testing was performed by the same investigator before and after training.

Isometric strength testing. Bilateral isometric muscle strength of the elbow flexors was determined by maximal voluntary contraction (MVC). Testing was conducted on 3 days separated by 24–48 h before training and on 2 days separated by 24–48 h after training. Testing was done on a custom-made preacher bench and strain gauge (model 32628CTL, Lafayette Instrument, Lafayette, IN). Subjects sat erect on the bench, with chest firmly pressed against the chest pad, legs fully extended, heels on the floor, toes pointed up, and resting arm placed palm up on the ipsilateral leg. The wrist of the arm being tested was placed between the arm pads, such that the medial epicondyle was aligned with the axis of rotation of the bench and the elbow joint formed a 90° angle, verified by measurement with a goniometer. The subject was instructed to produce a gradual buildup of maximum strength that was sustained for 3 s followed by a 1-min rest period. This process was repeated for a total of three trials. If the three trials were not within 5 ft/lb of each other, a maximum of six trials were performed. The three closest values within 5 ft/lb were averaged and recorded. The same investigator performed the MVC testing before and after training.

Resistance-training program. All subjects participated in a 12-wk, 2 days/wk upper arm, unilateral resistance program. All training was performed on the nondominant arm. Training sessions were supervised and lasted ~45–60 min. Each training session began with a warm-up consisting of two sets of 12 repetitions of the biceps preacher curl and seated overhead triceps extension. Five exercises were done in the following order: biceps preacher curl, seated overhead triceps extension, biceps concentration curl, triceps kickback, and standing biceps curl. Initial training weight was set at 65% of 1 RM. The subjects rested for 2 min between each set. Training was periodized to maximize muscle strength gains. Visits 1–8 required three sets of 12 repetitions at 65–75% of 1 RM, visits 9–18 required three sets of eight repetitions at 75–82% of 1 RM, and visits 19–24 required three sets of six repetitions at 83–90% of 1 RM.

MRI. CSA of the biceps brachii was determined bilaterally using MRI with 1.5-T systems. MRI was done before training and within 48–96 h of the final training session. Before imaging, maximum arm circumference or point of measurement was ascertained and marked with a radiographic bead (Beeckley Spots, Beeckley, Bristol, CT). The point of measurement was visually determined with the subject’s arm abducted 90° at the shoulder joint, palm supinated and open, and elbow flexed at 90°. The subject was then instructed to maximally flex the biceps muscles. The point of measurement was located, the skin was marked, and the tip of a radiographic bead was aligned and placed on the mark. The same investigator measured the point of measurement before and after training.

The MRI involved imaging a 24-cm length of the upper arm using 15 axial slices. Subjects were laid supine on the imaging bed, with the arm aligned to the isocenter of the magnet. The hand was placed in the anatomic position and affixed with tape to the scanner bed surface. Coronal and sagittal scout images were generated to locate the long axis of the humerus and to align the eighth axial slice with the point of measurement. With the point of measurement as the center point, 15 spoiled gradient images were taken (TE = 1.9 ms, TR = 200 ms, flow artifact suppression, 30° flip angle). Axial imaging began at the superior portion of the arm and proceeded distally toward the elbow joint. Each image slice was 16 mm thick, with a 0-mm interslice gap, 256 × 192 matrix resolution, 22 × 22 cm field of view, and number of experiments = 6. MRI data from all investigational sites were submitted to the central imaging facility at Yale University via Magneto Optical Disk or CD-ROM for further analysis. Images were analyzed using a custom-designed program created to function within Matlab (Math Works, Natick, MA). The eighth slice was the location analyzed for biceps CSA before and after training.

Genotyping methods. Genotyping was done to identify CNTF 1357 G → A (rs1800169) genotypes using TaqMan allele discrimination assays that employed the 5′-nuclease activity of Taq polymerase to detect a fluorescent reporter signal generated during the PCR. CNTF 1357 G → A alleles were detected simultaneously using allele-specific oligonucleotides labeled with different fluorophores, and genotypes were determined by the ratio of the two fluorophores used. Allele-specific PCRs for each SNP included 10 ng of genomic DNA, 900 nM forward (5′-GGTGATGACAGAAGATGTGGTGTT-3′) and reverse (5′-AGTC-CAGTTGATGTTCTTGTTAG-3′) PCR primer, 200 nM fluorescent allele discrimination probe [VIC-5′-TTCTGTATCTCCTGGCCAG-3′ (G allele) and FAM-5′-TTCTGTATCTCCTAGCCCCAG-3′ (A allele)], and TaqMan Universal PCR Master Mix, No AmpErase UNG (Applied Biosystems, Foster City, CA) in a final volume of 25 μL. The PCR profile was 10 min at 95°C (denaturation) and 44 cycles of 15 s at 92°C and 1 min at an annealing temperature of 60°C. Reactions were set up using an
and women

**Table 1. Subject characteristics by CNTF genotype for men and women**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Women (n = 335)</th>
<th>GA/AA (n = 117)</th>
<th>Men (n = 217)</th>
<th>GA/AA (n = 85)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>23.5±0.3</td>
<td>24.4±0.6</td>
<td>25.3±0.6</td>
<td>24.2±1.0</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>65±0.6</td>
<td>64.9±1.1</td>
<td>80.2±1.0</td>
<td>78.9±1.6</td>
</tr>
<tr>
<td>Height, cm</td>
<td>165±0.3</td>
<td>164.6±0.6</td>
<td>178±0.4</td>
<td>170±0.7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.1±0.2</td>
<td>23.9±0.4</td>
<td>25.2±0.3</td>
<td>25.0±0.5</td>
</tr>
</tbody>
</table>

Values are means ± SE. CNTF, ciliary neurotrophic factor; A, CNTF A1357 allele; G, CNTF G1357 allele; BMI, body mass index.

**DISCUSSION**

The present study is one of the first to examine the influence of the CNTF 1357 G → A SNP on the muscle strength response to resistance training among a large sample of healthy, young Caucasian adults. The most noteworthy finding is that the CNTF 1357 G → A SNP appears to make only a small contribution to the interindividual muscle strength
response to resistance training and that these associations are sex specific. Women with the CNTF G1357G genotype gained significantly more absolute 1-RM strength in the trained arm than carriers of the CNTF A1357 allele pre- to postraining. However, no significant genotype associations were observed in the trained arm in men. In women only, these results support our hypothesis that individuals carrying the A1357 allele would exhibit significantly lower gains in muscle strength after completion of a 12-wk resistance-training program than individuals homozygous for the G allele.

CNTF is a signaling molecule that has neurotrophic and myotrophic roles (5) in tissues such as motor neurons and skeletal muscle (9). Previous cross-sectional studies report genetic variation within the CNTF gene, specifically the CNTF 1357 G → A SNP, as a contributor to the interindividual variation in muscle strength among adults (1, 3, 15). However, only a few studies have incorporated resistance-training programs to examine associations among gene variants and muscle strength responses to resistance-training programs (2, 4, 7, 10, 13, 21). Our research team has demonstrated that 1-RM strength gains from the resistance-training intervention described in this study ranged from 0 to +250% (0 to +10.2 kg) and MVC changes in response to training ranged from −32 to +149% (−15.9 to +52.6 kg). Thus men and women exhibit a wide range of muscle strength responses to resistance training (8). Our findings demonstrate that the CNTF 1357 G → A SNP can account for a small amount of this variability. The exact mechanism explaining the increase in 1-RM strength in women homozgyous for the G1357G genotype is unclear. Guillet and colleagues (6) suggested that muscle strength performance may be controlled by the interaction of CNTF and its receptor complex. Women who are carriers of the A1357 allele are expected to produce less functional protein than noncarriers of the A1357 allele, and evidence suggests that lower levels of CNTF are associated with lower muscular strength (6). Therefore, the expected lower levels of functional CNTF protein produced by carriers of the A1357 allele and the resulting loss of its neurotrophic and myotrophic effects may have contributed to the differences in MVC gain in response to the training program among our female CNTF genotype groups. Because no significant differences were observed in CSA among CNTF genotype groups in the present study, perhaps the differences are due to a greater extent to CNTF’s neurotrophic role leading to an increase in muscle quality in women homozygous for the G1357G genotype.

Our finding that women who were carriers of the CNTF A1357 allele gained less absolute and allometric MVC strength in the untrained arm than women homozygous for the CNTF G1357 allele pre- to postraining (P < 0.05) was unexpected. The precise mechanisms behind the influence of the CNTF 1357 G → A SNP on the contralateral effect of the muscle strength response to training in this study are unclear. However, they are similar to our published findings regarding angiotensin I-converting enzyme (ACE) and the muscle strength response to a 12-wk unilateral, upper arm resistance-training program (13). Our laboratory demonstrated a variable muscle strength response to training in the untrained arm among ACE ID genotype groups (13). We demonstrated that 1-RM strength gains were greater in carriers of the ACE D allele (ACE DD/ID) than in those with the ACE II genotype in the untrained arm and concluded that the ACE ID genotype is primarily associated with cross-education and learning effects, rather than the inherent muscle strength adaptations that result from resistance training.

Evidence suggests that high-force, unilateral, voluntary contractions may have an acute and potent effect on the efficacy of neural elements controlling the untrained limb (11). Lee and Carroll (11) indicate that it is possible that, with resistance training, long-lasting adaptations may be induced in neural circuits mediating these contralateral training effects. These researchers have hypothesized that unilateral resistance training may activate neural circuits that chronically modify the efficacy of motor pathways that project to the untrained limb. This projection of neural input to the opposite limb may subsequently lead to an increased capacity to drive the untrained muscles and, thus, result in increased muscle strength in trained and untrained limbs (11). Munn et al. (12) estimated that the effect of unilateral resistance training on maximal voluntary strength of the contralateral limb was a 7.8% increase above initial strength values. However, the contralateral effects of strength training reported in individual studies varied from −2.7 to 21.6% of initial strength (12). Given that CNTF has neurotrophic influences on motor neurons (9) and that variation within the CNTF gene have been associated with muscular performance, the results from the present study may allow for the generation of future hypotheses regarding this phenomenon. Further work is needed to more precisely delineate the mechanisms for the associations we observed.

The present study has several limitations. Because of the small number of individuals homozygous for the CNTF A1357 allele, it was necessary to combine specific genotype groupings; therefore, we were unable to examine a possible dose-response relationship among the three genotype groups. We only examined genetic variation within an SNP and its relationship with the muscle strength response to a resistance-training program. We found that CNTF 1357 G → A SNP explained only a small portion of the variability in the muscle strength response to training in women. Thus it is likely that the strength-training response to a resistance-training program involves multiple genes, many of which remain to be identified. Variation within the CNTF gene has also been associated with muscle strength (16), which was not examined in this study. Another limitation of the present study is that there is no
obvious explanation for the CNTF 1357 G → A polymorphism differentially affecting the training response between men and women. However, our group and other previously published candidate gene association studies involving skeletal muscle phenotypes have also observed sex-specific differences (2, 18, 22–26). We previously suggested that sex differences may be partially due to sex-specific hormonal differences between men and women (2). If we consider that genes do not act in a vacuum and that the hormonal environment is vastly different between men and women, as speculated by Walsh et al. (25), the sex differences that have been observed in these studies could partially be due to sex-specific gene × hormonal environment interactions.

The study of genomic factors contributing to the health-related benefits of exercise has increased dramatically over the past 10 years (14). Important reasons for undertaking work in exercise genomics is that 1) exercise scientists can use genetic information to identify physiological pathways underlying the responses and adaptations to exercise and 2) clinicians and sports medicine professionals will ultimately be able to use genetic information to individualize exercise prescriptions to maximize the use of exercise as a therapeutic option in the prevention, treatment, and management of chronic diseases and conditions such as sarcopenia. The present results add the CNTF 1357 G → A polymorphism to a growing list of SNPs that have been tentatively identified as making small contributions to interindividual variation in muscle strength gains in response to training (for more information on muscle strength genes see Ref. 1a). Potential mechanisms for these findings are unclear; therefore, further research is warranted to ascertain the physiological pathways by which the CNTF 1357 G → A polymorphism modulates the muscle strength response to training in women. In conclusion, these results suggest that the CNTF locus may contribute to the interindividual variation observed in the muscle strength response to training in women, but not in men.

ACKNOWLEDGMENTS

We thank the students and technicians at the participating institutions for their time and effort and the subjects for their participation and commitment to the project.

GRANTS

This study was supported by National Institutes of Health Grant NINDS/NIAMS/NS-40606 and the Parsons Family Foundation.

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