Polymorphisms in the CNTF and CNTF receptor genes are associated with muscle strength in men and women

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De Mars G, Windelinckx A, Beunen G, Delecluse C, Lefevre J, Thomis MA. Polymorphisms in the CNTF and CNTF receptor genes are associated with muscle strength in men and women. J Appl Physiol 102: 1824–1831, 2007. First published February 1, 2007; doi:10.1152/japplphysiol.00692.2006.—Genotypic associations between polymorphisms in the ciliary neurotrophic factor (CNTF) and CNTF receptor (CNTFR) genes and muscular strength phenotypes in 154 middle-aged men (45–49 yr) and 138 women (38–44 yr) and 99 older men (60–78 yr) and 102 older women (60–80 yr) were tested to validate earlier association studies. Allelic interaction effects were hypothesized between alleles of CNTF and CNTFR. We performed analysis of covariance with age, height, and fat-free mass (FFM) as covariates. FFM was anthropometrically estimated by the equation of Durnin-Womersley. Isometric, concentric, and eccentric torques for the knee flexors (KF) and extensors (KE) were measured using Biodex dynamometry. In the older male group, T-allele carriers of the C-1703T polymorphism in CNTFR performed significantly better on all noncorrected KF torques, whereas only noncorrected KE isometric torque at 120° and concentric torque at 240°/s were higher than the C/C homozygotes (P < 0.05). When age, height, and FFM were used as covariates, T-allele carriers performed only better on KE and KF isometric torque at 120° (P < 0.05). Concentric KE torque at 180°/s was lower in middle-aged female A-allele carriers compared with the T/T subjects for the T1069A polymorphism in CNTFR. After correction for age, height, and FFM, middle-aged female A-allele carriers exhibited lower values on all concentric KE strength measures and isometric torque at 120°. There was a lack of association with the CNTF G-6A polymorphism in men, with inconclusive results for a limited number of phenotypes in women. No significant CNTF/ CNTFR allele interaction effects were found. Results indicate that CNTFR C-1703T and T1069A polymorphisms are significantly associated with muscle strength in humans.

ciliary neurotrophic factor; association analysis; peak torque; aging; sex-specific differences

FROM A GENERAL HEALTH PERSPECTIVE, muscular fitness is associated with performing activities of daily living (ADLs) independently (17). Indicators of functional status of skeletal muscle (strength, power, and endurance) are positively associated with bone health (25) and psychological well-being (22) and negatively associated with falls and fractures (28), morbidity (24) and mortality (10, 21). The age-associated decline in muscular strength and mass may also be related to prolonged disuse and/or chronic disease, such that a vicious cycle is created wherein inactivity leads to sarcopenia, which further worsens the ability to perform ADLs. Results of these studies raise the question of which genes, besides environmental factors like nutrition, social status, and training, influence musculoskeletal fitness components that are determining factors for predicting health status, particularly in the elderly. Several studies suggest that muscle cross-sectional area (MCSA) and isometric (Fisom), concentric (Fconc), and eccentric (Fecc) muscle strength are under moderate to high genetic control with heritabilities ranging between 60 and 95% for MCSA, 44 and 78% for Fisom, 31 and 61% for Fconc, and 65 and 77% for Fecc (18, 32–37). A first step in the screening of subjects exhibiting low muscle strength levels is the detection of polymorphisms responsible for muscle strength characteristics via association studies.

In the present study, four polymorphisms in ciliary neurotrophic factor (CNTF) and its receptor (CNTFR) are validated against earlier reports on their association with muscle strength (2, 26, 27). Furthermore, we explore allelic interaction effects between both CNTF and CNTFR gene variants.

CNTF

Takahashi et al. (31) reported a G-6A null mutation at 6 bp downstream of exon 2 in the CNTF gene that generated a new splice acceptor site in 391 Japanese subjects. Although this resulted in the expression of a mutant mRNA, no detectable amounts of mutated CNTF protein were produced after immunoblot analysis, indicating that the mutated protein may be very unstable and rapidly degrades after translation.

Roth et al. (27) examined the relationship between the G-6A mutation (rs1800169) and muscle strength in 494 healthy men and women (413 Caucasians, 63 African-Americans, and 18 of other races) across the entire adult age span (20–90 yr). Concentric and eccentric peak torques were measured at angular velocities of 30°/s and 180°/s for the dominant knee flexors (KF) and extensors (KE). Maximal voluntary isometric torque was measured for the KE at 120°. In contrast to their hypothesis, they demonstrated that individuals heterozygous for the CNTF null (A allele) mutation (G/A) exhibited significantly higher concentric peak torque of the KE and KF at 180°/s than G/G homozygotes when age, sex, and body mass were covaried. When the dominant leg fat-free mass (FFM) was used as a covariate, concentric peak torque of the KE at 180°/s was also significantly greater in the G/A group. Similar results were found in a subanalysis of subjects 60 yr and older, as well as in Caucasian subjects. In contrast, A/A homozygotes...
demonstrated significantly lower eccentric peak torque at 30°/s for both KE and KF compared with G/G and G/A groups.

The same group (4) assessed surface-detected motor unit potential (SMUP) area and mean firing rates (mFR) from motor units in the human vastus medialis muscle resulting in different responses between G/G vs. G/A genotypes in 36 women and men (aged 30 to 94 yr) from the Baltimore Longitudinal Study of Aging (BSA) and a cohort of 33 young and older men and women (aged 23 to 73 yr). The CNTF G/A genotype was associated with smaller SMUP area motor units and lower mFR at higher force levels and fewer but larger units at lower force levels than G/G homozygotes. At higher force levels, G/A subjects generated more force per motor unit size, suggesting more efficient motor unit function with increasing muscle force.

Arking et al. (2) identified five single-nucleotide polymorphisms (SNPs) significantly associated with grip strength in 363 Caucasian, community-dwelling women aged 70 to 79 yr after correction for age, body mass index (BMI), and osteoarthritis. Haplotype analysis was performed, and a single haplotype type associated with grip strength was identified. The G-6A null allele fully explained the association between this haplotype and grip strength, with individuals homozygous for the null allele exhibiting a 3.8-kg lower grip strength.

The neuroregulatory cytokine CNTF has neurotrophic and myotrophic characteristics and is expressed in Schwann cells (1, 8, 20, 30). Daily injection of CNTF and neurotrophin (NT)-3 or NT-4 (20) or recombinant CNTF and human CNTF (12) attenuated the denervation-induced atrophy and motor neuron loss in both slow and fast muscles after neonatal axotomy in rats. CNTF treatment prevented cell death of the spinal nucleus of the bulboconusversus, which leads to an increased volume of the levator ani muscle that was significantly greater than in vehicle controls (8, 23). Sciatic nerve CNTF levels are positively associated with swimming performance and muscular strength in rats (11). In addition, exogenous CNTF administration in older rats attenuated the age-associated decline in muscle strength.

**CNTFR**

The CNTFR is composed of three components: 1) a CNTFR-α subunit, 2) the IL-6 receptor gp130β subunit, and 3) the leukemia-inhibitory factor receptor-β subunit (5, 14, 29). Upregulation of CNTFR expression in skeletal muscle was demonstrated in studies investigating hindlimb unweighting (9) and muscle damage in rats (16), in patients with peripheral neuropathies (15), and during denervation in human skeletal muscle (38).

Three newly identified polymorphisms in the CNTFR gene were genotyped by Roth et al. (26). T-allele carriers of the C174T polymorphism in exon 9 exhibited greater total and lower limb FFM and significantly higher KE concentric and eccentric peak torque at 30°/s and 180°/s when corrected for age, height, physical activity, and race. However, after adjustment for lower limb FFM, the group differences in the strength measures were no longer statistically significant. No association was found between the strength-related phenotypes and the C-1703T polymorphism in the 5′-promoter region and the T1069A polymorphism in intron 5 of CNTFR. Effects of these polymorphisms on receptor expression levels in humans have not been described in literature.

In this study we try to elucidate the inconclusive results on G-6A polymorphism effects on strength as reported by Roth et al. (27) and Arking et al. (2). More specifically, on the basis of functional data of the Takahashi et al. (31) study, we test whether rare allele homozygotes (AA) and heterozygotes (GA) of the G-6A polymorphism in intron 1 at 6 bp downstream of exon 2 in CNTF exhibit significantly lower knee strength and FFM than CNTF wild-type/GG homozygotes using three genotype group comparison tests. In the CNTFR gene, we try to replicate the Roth et al. (26) finding that T-allele carriers of the C174T polymorphism in exon 9 express higher strength levels compared with C-allele carriers. Furthermore, the C-1703T polymorphism in the 5′-promoter region and T1069A polymorphism in intron 5 of CNTFR are studied to explore their role in human muscle strength variation [negative findings by Roth et al. (26)]. As a unique follow-up on the initial association findings, allelic interaction effects between the CNTF and CNTFR polymorphisms are investigated in the four different age and sex subgroups and in the total group of men and women separately.

**MATERIALS AND METHODS**

**Subjects**

The sample for this study was composed of two Caucasian cohorts, conducted in the framework of the Policy Research Centre Sport, Physical Activity, and Health. In 2002–2004, 154 healthy middle-aged men (45–49 yr) and 138 healthy women (38–44 yr) participated in a longitudinal follow-up phase of the Leuven Longitudinal Study on Lifestyle, Physical Fitness, and Health. The second study cohort was composed of 99 older men (60–78 yr) and 102 older women (60–80 yr) who were recruited to participate in a training intervention study through newspaper advertisements, fliers, regional television programs, and meeting groups in the region of Leuven, Belgium. Their ethnicity was questioned by birthplace and country of origin up to two generations. All subjects were of Caucasian origin. Subjects had to be free from diseases or medications known to affect bone metabolism or muscle strength and underwent medical screening by a physician. Older subjects who participated in endurance or strength training or who were physically active for more than 2 h/wk at moderate intensity were excluded. Only baseline values were retained for further analyses.

All subjects gave their written informed consent after being explained the procedures and purpose of each study. Both studies were approved by the medical and ethical committee of the Katholieke Universiteit Leuven.

**Measurements**

**Body composition.** An extended set of skinfolds, length, width, and circumference measurements were taken from all subjects by an experienced anthropometrist in standardized conditions (13). FFM was estimated by the equation of Durnin-Womersley (39) for which transversal knee-joint axis and connected to the distal end of the tibia using a length-adjustable rigid lever arm. Drouin et al. (6) demonstrated that this instrument provided mechanically valid and reliable measures of torque. All tests were performed unilaterally on the right.
side, unless the right knee was injured. The subjects were seated on a backward inclined chair (15°) with the upper leg, hips, and shoulder stabilized using safety belts. Subjects were verbally encouraged to perform at their maximum effort, and visual feedback of their performance was presented after each test. The data were assessed by three highly trained evaluators. All performance curves were visually reviewed for quality control, e.g., to exclude peak torque measures with incorrect range of motion, or lack of a torque plateau in isometric contractions.

Maximal isometric knee extension strength was tested in two knee joint angles at 150° and 120° (180° is extended leg). The subject performed two isometric contractions in each angle lasting for 5 s, with a rest period of 10 s between the trials. The contractions in the different angles were separated by a 20-s rest interval. The highest torque (N·m) of the two trials was recorded as the maximal isometric strength. Maximal isometric knee flexion strength was only tested at 120°.

The subject performed two series of consecutive isokinet is extension-flexion movements against the lever arm with a determined velocity of 60°/s (4 repetitions) and 240°/s (6 repetitions) between a knee angle of 90° and 160°. Between the two series there was a rest period of 20 s. The maximal dynamic extension and flexion strength were determined as the peak torque (N·m) during these series of knee extension and flexion.

Maximal eccentric knee extension and flexion at 60°/s were tested with two series of consecutive flexion-extension movements against the lever arm. The maximal eccentric flexion and extension were retained for further analyses.

Finally, subjects performed a series of 25 consecutive extension and flexion movements between a knee angle of 90° and 160° with a determined velocity of 180°/s. Again, maximal dynamic extension and flexion strength at 180°/s were assessed as the peak torque (N·m) registered within the first three to five repetitions.

Laboratory Methods

Genomic DNA was prepared from EDTA whole blood by a standard salting-out method (19). The following SNPs selected on the basis of the studies of Roth et al. (26, 27) were genotyped: C174T in exon 9; C-1703T in the 5′-promoter region and T1069A in CNTFR (rs2070802) in intron 5, 37 bp upstream of exon 6 for CNTFR; and rs1800169 at position G-6A in intron 1 at 6 bp downstream of exon 2 for CNTF. Genotyping was determined at Genaissance Pharmaceuticals (New Haven, CT) via Sequenom’s MassARRAY platform.

Statistics

Statistical analyses were performed separately for men and women from each sample as well as for the combined groups (total group of men and total group of women). A χ²-test was used to determine deviations of genotype distribution from Hardy-Weinberg equilibrium. Log and square root transformations were executed to obtain normal distributions. Strength variables were analyzed as outcome variables with analysis of covariance (ANCOVA), with age, height, and FFM as covariates.

The main goal of this study is to validate the results of Roth et al. (26, 27) and Arking et al. (2) in a different population of male and female middle-aged and older subjects. In addition, interaction effects between CNTF and the CNTFR polymorphisms were tested using preplanned contrasts with a mixed procedure in SAS version 9.1. Different interaction hypotheses were formulated on the basis of gene-specific association findings or trends within each study cohort. Given small minor allele frequencies in each polymorphism, rare allele carrier groups were considered. In the older male cohort and the total group of men, we hypothesized that subjects carrying the A allele in G-6A of CNTF and homozygote for the C allele in C-1703T of CNTFR would produce less strength than G/G homozygotes and T carriers, respectively. Furthermore, in the middle-aged men A-allele carriers of G-6A in combination with the rare T allele of C-1703T are expected to produce less strength than G/G and C/C homozygotes, respectively. Additionally, in the male cohorts and total group, T carriers in C174T of CNTFR in combination with the rare A allele in G-6A are expected to exhibit more strength than the C/C and G/G homozygotes, respectively. In the three female cohorts, the presence of an A allele in G-6A in combination with an A allele in T1069A in CNTFR was expected to produce lower strength levels than the G/G and T/T homozygotes, respectively. Significance level was set at 0.05. Explained variance (r²) of the polymorphisms was quantified by comparison of the ANCOVA model with and without the SNP as factor.

RESULTS

One polymorphism in the CNTF gene (G-6A in intron 1) and three polymorphisms in the CNTFR gene (C-1703T in the 5′-promoter region, T1069A in intron 5, 37 bp upstream of exon 6, and C174T in exon 9) were genotyped. Genotype frequencies and number of subjects successfully genotyped per study sample are shown in Table 1; χ²-tests revealed no deviations from Hardy-Weinberg equilibrium for the genotypes studied (P values > 0.01). No association of CNTF or CNTFR polymorphisms with age, body height, BMI, or FFM was found except for C174T where T-allele carriers (n = 28) in the middle-aged male sample are taller than homozygotes (n = 124) for the C allele (180.99 ± 1.04 vs. 177.05 ± 0.49 cm).

Table 1. Genotype frequencies for polymorphisms in CNTF and CNTFR

<table>
<thead>
<tr>
<th>Genotype Frequencies, %</th>
<th>Group 11</th>
<th>Group 12</th>
<th>Group 22</th>
<th>n</th>
<th>HWE P Value</th>
</tr>
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<tbody>
<tr>
<td>G-6A, intron 1</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Middle-aged men</td>
<td>73</td>
<td>23</td>
<td>4</td>
<td>154</td>
<td>0.18</td>
</tr>
<tr>
<td>Older men</td>
<td>76</td>
<td>23</td>
<td>1</td>
<td>98</td>
<td>0.37</td>
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<tr>
<td>Total cohort men</td>
<td>74</td>
<td>23</td>
<td>3</td>
<td>252</td>
<td>0.42</td>
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<tr>
<td>Middle-aged women</td>
<td>77</td>
<td>21</td>
<td>2</td>
<td>137</td>
<td>0.72</td>
</tr>
<tr>
<td>Older women</td>
<td>79</td>
<td>19</td>
<td>2</td>
<td>101</td>
<td>0.65</td>
</tr>
<tr>
<td>Total cohort women</td>
<td>78</td>
<td>20</td>
<td>2</td>
<td>238</td>
<td>0.77</td>
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<tr>
<td>C-1703T, 5′ promoter</td>
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<td>Middle-aged men</td>
<td>60</td>
<td>35</td>
<td>5</td>
<td>154</td>
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<tr>
<td>Older men</td>
<td>62</td>
<td>34</td>
<td>4</td>
<td>98</td>
<td>0.69</td>
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<tr>
<td>Total cohort men</td>
<td>61</td>
<td>35</td>
<td>4</td>
<td>252</td>
<td>0.69</td>
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<tr>
<td>Middle-aged women</td>
<td>59</td>
<td>37</td>
<td>4</td>
<td>137</td>
<td>0.53</td>
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<tr>
<td>Older women</td>
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<td>4</td>
<td>101</td>
<td>0.52</td>
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<tr>
<td>Total cohort women</td>
<td>60</td>
<td>36</td>
<td>4</td>
<td>238</td>
<td>0.44</td>
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<td>T1069A, intron 5</td>
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<td>Middle-aged men</td>
<td>79</td>
<td>20</td>
<td>1</td>
<td>153</td>
<td>0.75</td>
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<tr>
<td>Older men</td>
<td>69</td>
<td>31</td>
<td>0</td>
<td>98</td>
<td>0.04</td>
</tr>
<tr>
<td>Total cohort men</td>
<td>75</td>
<td>24</td>
<td>1</td>
<td>251</td>
<td>0.17</td>
</tr>
<tr>
<td>Middle-aged women</td>
<td>73</td>
<td>23</td>
<td>4</td>
<td>137</td>
<td>0.27</td>
</tr>
<tr>
<td>Older women</td>
<td>77</td>
<td>20</td>
<td>3</td>
<td>101</td>
<td>0.36</td>
</tr>
<tr>
<td>Total cohort women</td>
<td>75</td>
<td>21</td>
<td>3</td>
<td>238</td>
<td>0.11</td>
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<tr>
<td>C174T, exon 9</td>
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<td></td>
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<td>82</td>
<td>17</td>
<td>1</td>
<td>152</td>
<td>0.77</td>
</tr>
<tr>
<td>Older men</td>
<td>80</td>
<td>17</td>
<td>3</td>
<td>96</td>
<td>0.15</td>
</tr>
<tr>
<td>Total cohort men</td>
<td>81</td>
<td>17</td>
<td>2</td>
<td>248</td>
<td>0.19</td>
</tr>
<tr>
<td>Middle-aged women</td>
<td>84</td>
<td>16</td>
<td>0</td>
<td>137</td>
<td>0.13</td>
</tr>
<tr>
<td>Older women</td>
<td>90</td>
<td>10</td>
<td>0</td>
<td>101</td>
<td>0.12</td>
</tr>
<tr>
<td>Total cohort women</td>
<td>87</td>
<td>13</td>
<td>0</td>
<td>238</td>
<td>0.13</td>
</tr>
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</table>

Groups 11, 12, and 22 represent the genotype groups G/G, G/A, and A/A for the G-6A polymorphism; C/C, C/T, and T/T for both C-1703T and C174T; and T/T, A/T, and A/A for T1069A, respectively; n, no. of subjects successfully genotyped per study sample. CNTF, ciliary neurotrophic factor; CNTFR, CNTF receptor. P values represent χ²-analyses to test for deviation from Hardy-Weinberg equilibrium (HWE).
Similar results were found for the C-1703T in the older sample where T-allele carriers (n = 37) were taller than C/C homozygotes (n = 61) (175.42 ± 0.97 vs. 171.91 ± 0.76 cm; P = 0.0062). In the total group of women, female T-allele carriers have more FFM (44.61 ± 0.31 kg; n = 230) than A/A homozygotes (40.91 ± 1.63 kg; n = 8) for the T1069A polymorphism in intron 5 (P = 0.0271). The power to test phenotypic differences between homozygous rare allele and other genotype groups is limited. Comparisons for CNTFR polymorphisms are therefore based on dominance models between rare allele carriers (both homo- and heterozygotes) and homozygote wild-type individuals (see Table 3). CNTF G-6A genotype group differences are studied to be able to compare our results with those from Roth et al. (27) and Arking et al. (2) (Table 2).

**G-6A in CNTF (Intron 1)**

Genotypic association results are reported in Table 2. Female middle-aged A/A homozygotes produced less KF concentric strength at 180°/s with and without correction for age, height, and FFM (P = 0.0471 and 0.0453, respectively). In the older female group, G/A heterozygotes performed well on isometric KE strength at 150° flexion than both G/G and A/A homozygotes after adjustment for covariates (P = 0.0229). In men, no associations could be found.

**C-1703T in CNTFR (Promoter)**

In the older male group, T-allele carriers performed significantly better on all noncorrected KF torques, whereas only noncorrected KE isometric torque at 120° and concentric torque at 240°/s were higher than the C/C homozygotes (P < 0.05) (Table 3). When age, height, and FFM were used as covariates, T-allele carriers performed only better on KE and KF isometric torque at 120° (P < 0.05). Middle-aged men carrying the C allele produced less concentric KF torque (64.62 ± 1.32 N·m; n = 147) than the T/T group (81.40 ± 6.15 N·m; n = 7) (P = 0.0224). To improve power, association analyses were performed on the total group of men (middle aged + older) with correction for age, height, and FFM. C carriers (57.13 ± 0.99 N·m; n = 241) exhibited less KF concentric torque at 240°/s than the T/T group (69.00 ± 4.83 N·m; n = 11) (P = 0.0183). The variance explained by polymorphism C-1703T ranged from 5 to 9%. No significant effects were found in the female groups.

**T1069A in CNTFR (Intron 5)**

Concentric KF torque at 180°/s was lower in middle-aged female A-allele carriers compared with the T/T subjects. After correction for age, height, and FFM, middle-aged female A-allele carriers exhibited lower values on the concentric KF strength measures and isometric torque at 120° (Table 3). In the older female group, T-allele carriers (n = 98) performed better on KF concentric torques at 60°/s (45.91 ± 1.13 vs. 35.74 ± 6.16 N·m; P = 0.03), 180°/s (41.62 ± 0.88 vs. 32.70 ± 4.90 N·m; P = 0.04), and 240°/s (27.23 ± 0.75 vs. 20.21 ± 3.94 N·m; P = 0.04) than the A/A homozygotes (n = 3), whereas the group differences in these torque measures were no longer statistically significant after adjustment for age, height, and FFM. The T1069A polymorphism explained 2–9% of the total variance.

### Table 2. Peak torque values for the knee extensors and flexors for different genotypes in CNTF

<table>
<thead>
<tr>
<th>Subj. n</th>
<th>Isometric (180°) N·m</th>
<th>Concentric (60°) N·m</th>
<th>Concentric (120°) N·m</th>
<th>Concentric (180°) N·m</th>
<th>Concentric (240°) N·m</th>
<th>KF Extensors (60°) N·m</th>
<th>KF Extensors (180°) N·m</th>
<th>KF Extensors (240°) N·m</th>
<th>KF Flexors (60°) N·m</th>
<th>KF Flexors (180°) N·m</th>
<th>KF Flexors (240°) N·m</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GG</strong></td>
<td>63.30 ± 62</td>
<td>65.13 ± 56</td>
<td>117.18 ± 68</td>
<td>112.98 ± 58</td>
<td>61.80 ± 43</td>
<td>72.7 ± 6</td>
<td>32.6 ± 8</td>
<td>112.98 ± 58</td>
<td>72.7 ± 6</td>
<td>32.6 ± 8</td>
<td></td>
</tr>
<tr>
<td><strong>GA</strong></td>
<td>60.38 ± 62</td>
<td>62.67 ± 56</td>
<td>112.98 ± 68</td>
<td>112.98 ± 58</td>
<td>61.80 ± 43</td>
<td>72.7 ± 6</td>
<td>32.6 ± 8</td>
<td>112.98 ± 58</td>
<td>72.7 ± 6</td>
<td>32.6 ± 8</td>
<td></td>
</tr>
<tr>
<td><strong>AA</strong></td>
<td>60.38 ± 62</td>
<td>62.67 ± 56</td>
<td>112.98 ± 68</td>
<td>112.98 ± 58</td>
<td>61.80 ± 43</td>
<td>72.7 ± 6</td>
<td>32.6 ± 8</td>
<td>112.98 ± 58</td>
<td>72.7 ± 6</td>
<td>32.6 ± 8</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE, all adjusted for age, height, and fat-free mass (FFM). Adjusted P-values are corrected for age, height, and FFM. *significant difference from A/A, G/G significantly different from A/A.
ASSOCIATION BETWEEN CNTF/CNTFR AND MUSCLE STRENGTH

Older Men CNTFR C-1703T Middle-Aged Women CNTFR T1069A Middle-Aged Men CNTFR C174T

Adjusted Unadjusted Adjusted

T/T C/T C/C T/T C/T C/C

P Value P Value

\begin{tabular}{|c|c|c|c|c|}
\hline
Subjects, n & 37 & 61 & 36 & 101 & 28 & 124 \\
\hline
Knee extensors & & & & & & \\
\hline
Isometric (150°), N & 85.14 ± 3.13 & 185.28 ± 5.63 & 78.85 ± 2.96 & 110.00 ± 1.62 & 81.51 ± 2.76 & 87.89 ± 2.23 \\
\hline
Isometric (120°), N & 176.84 ± 5.63 & 376.52 ± 11.84 & 157.51 ± 7.81 & 224.71 ± 11.85 & 164.74 ± 5.63 & 154.39 ± 5.01 \\
\hline
Concentric (240°/s), N & 240.38 ± 11.85 & 480.76 ± 23.70 & 210.00 ± 11.85 & 280.00 ± 11.85 & 220.00 ± 11.85 & 250.00 ± 11.85 \\
\hline
Eccentric (60°/s), N & 219.00 ± 11.85 & 438.00 ± 23.70 & 189.00 ± 11.85 & 258.00 ± 11.85 & 208.00 ± 11.85 & 238.00 ± 11.85 \\
\hline
Knee flexors & & & & & & \\
\hline
Isometric (120°), N & 111.88 ± 3.04 & 223.76 ± 6.08 & 100.25 ± 3.04 & 180.00 ± 3.04 & 90.00 ± 3.04 & 150.00 ± 3.04 \\
\hline
Concentric (240°/s), N & 132.25 ± 4.14 & 264.50 ± 8.28 & 123.36 ± 4.14 & 246.75 ± 8.28 & 120.00 ± 4.14 & 240.00 ± 8.28 \\
\hline
\end{tabular}

Values are means ± SE, all adjusted for age, height, and FFM. Adjusted values are corrected for age, height, and FFM. *T carriers significantly different from C/C homozygotes. †A carriers significantly different from T/T homozygotes.

In the middle-aged male group, T-allele carriers exhibited significantly lower KE eccentric torque at 60°/s than C/C homozygotes (P = 0.0223). This result was still significant after correction for age, height, and FFM (P = 0.013). KE isometric torque at 150° was lower for T carriers compared with C/C homozygotes after correction for age, height, and FFM (P = 0.0065). No association was found with KE phenotypes (Table 3).

C-allele carriers exhibited lower KF concentric strength at 240°/s (64.91 ± 1.27 N·m; n = 150) than T/T homozygotes (105.38 ± 10.39 N·m; n = 2) with (P = 0.001) and without correction for age, height, and FFM (P = 0.003). In the total group of men (middle aged + older men), T carriers exhibited lower KE isometric torque at 150° and eccentric torque at 60°/s compared with the C/C homozygotes (80.89 ± 2.90 vs. 89.46 ± 3.19 N·m; P = 0.009, n = 47; and 195.42 ± 7.46 vs. 216.29 ± 3.55 N·m, P = 0.01, n = 201, respectively). In the total cohort of men and women, C/C homozygotes (n = 407) performed better on isometric KE torque at 150° (75.82 ± 0.89 N·m; P = 0.0057) and 120° (153.67 ± 5.41 N·m; P = 0.0193) than T carriers (n = 78) (69.05 ± 2.10 and 133.97 ± 12.63 N·m, respectively). C-allele carriers (n = 449) exhibited lower KF concentric torque at 240°/s (44.33 ± 0.69 N·m) than T/T homozygotes (64.85 ± 3.48 N·m; n = 5) (P = 0.001). The variance explained by polymorphism C174T ranged from 1 to 4%.

No allele × allele interactions were found between the CNTF polymorphism and the three CNTFR SNPs in any of the study cohorts on any of the strength measurements. Because of the small sample size in the middle-aged male group, interactions between the G-6A and C-1703T polymorphisms could not be tested.

DISCUSSION

Earlier reported findings on a CNTF gene polymorphism and muscular fitness have been ambiguous (26, 27), and association with CNTFR polymorphisms are only limited to one study (26). The purpose of the present study was therefore to elucidate these associations in an independent population of middle-aged and senior men and women and to study interaction between CNTF and CNTFR alleles. Two major trends could be demonstrated for the CNTFR polymorphisms (C-1703T and T1069A).

CNTFR C-1703T

First, older male T-allele carriers of the C-1703T polymorphism in CNTFR produce more KF strength (isometric, concentric, and eccentric) and KE isometric torque at 120° and concentric torque at 240°/s than C/C homozygotes (Table 3). Genotype effects were only significant for KF and KE isometric torques when accounting for age and body size covariates. This might be accounted for by the older male T allele carriers who are taller than C/C homozygotes and thus have a longer force arm (lower leg). Although this polymorphism, located in the 5′-promoter region, is not translated into protein, it might be involved in the regulation of CNTFR expression. The
presence of a T allele might induce higher gene expression levels of the CNTFR in muscle compared with the presence of an A allele, which could lead to higher force generation. Further research is needed with focus on the relationship between CNTFR polymorphisms, gene expression levels, and muscle strength in humans. Guillet et al. (11) observed a 10- to 20-fold increase in CNTFR mRNA and a twofold to fourfold decrease in CNTF expression in aged rats. They hypothesized that upregulation of CNTFR expression might be involved in a compensatory phenomenon to maintain a CNTF response similar to that present in adult animals. Roth et al. (26), however, did not find any association between the C-1703T polymorphism and the strength variables studied. Results of both studies are difficult to compare as the present study cohort consisted of older Caucasian people (60 to 78 yr), while the age of subjects in the study of Roth et al. (26) ranged from 20 to 91 yr and was a mixture of Caucasians and African-Americans. More studies in larger sample sizes and different age categories and ethnicities and in both sexes should be conducted to elucidate age-, sex-, and ethnicity-by-genotype interactions.

In the middle-aged cohort, a different dominance model led to significant results, with C-allele carriers producing less concentric strength at 240°/s than T/T homozygotes with \( P = 0.0155 \) and without correction \( P = 0.0224 \). Similar results were found in the total group of men \( P = 0.0183 \). These results are in line with the dominance model comparing T-allele carriers vs. C/C homozygotes in the older male group. The presence of a T allele seems to result in higher levels of force production than the presence of a C allele.

**CNTFR T1069A**

Second, we demonstrated that female middle-aged A-allele carriers of the T1069A polymorphism located in intron 5 of CNTFR performed significantly worse on all KE strength phenotypes when corrected for age, height, and FFM \( P < 0.05 \), except for eccentric torque (Table 3). Possible linkage disequilibrium (LD) of this noncoding SNP with a nearby functional exonal SNP might regulate this association signal. Again, Roth et al. (26) did not find any association for this polymorphism and their strength phenotypes.

**CNTFR C174T**

In our study, middle-aged men carrying the T allele in the C174T polymorphism of CNTFR exhibited lower eccentric and isometric KE torques (Table 3). The latter being significant only after adjustment for age, height and FFM \( P < 0.05 \). Similar results were found when the older men were added to the analyses. However, in the study of Roth et al. (26), men carrying the T allele produced significantly more KE eccentric peak torque at slower (30°/s) and higher (180°/s) speeds as well as higher isometric KE torques at 120° than C/C homozygotes only when covaried for age, race, height, and physical activity. They found the same results for women, except for isometric peak torque. However, when lower limb FFM was added as a covariate, all association findings became nonsignificant. Therefore, Roth et al. concluded that the C174T polymorphism association was caused by its effect on fat free mass. To conform more closely to the Roth et al. setup, we performed an additional analysis on the total group of men and women \( n = 493 \) adjusted for sex, age, height, and FFM. No differences in genotype frequencies from Hardy-Weinberg equilibrium and no group differences for subject characteristics were observed. We therefore failed to replicate the findings of Roth et al. (26), who demonstrated significantly lower total FFM and lower limb FFM in common C-allele homozygotes compared with carriers of the rare T allele \((C/T + T/T)\) with age, sex, race, physical activity, and height covaried. The role of individual variability in physical activity in the BLSA sample is not quantified and can therefore not be judged as a factor in the dissimilarity of findings. In a younger male sample of the Leuven Genes for Muscular Strength Study, sports participation as measured by the Baekesports index did not contribute significantly to muscle strength variability (13). The results in the whole group analysis for strength measures pointed toward a better isometric KE performance in C/C homozygotes compared with T-allele carriers. Roth et al. (26) did not find associations with maximal isometric torque, and the C/C genotype group performed worse for KE concentric and eccentric strength (covaried for age, sex, height, physical activity, and race). Results of both studies are difficult to compare as physical activity was not considered as a covariate in our analyses, and FFM was regionally determined in the Roth et al. study as compared with a total body FFM estimate in our sample. Furthermore, our sample consisted of only Caucasians, while allele frequency differences between Caucasians and African-Americans \((T_{Cauc} = 0.11 \text{ vs. } T_{Afr-Am} = 0.20)\) are present in the BLSA study. More research is needed on larger samples to elucidate these discrepancies. The C174T polymorphism is located in exon 9 in the 3′-untranslated region and is therefore not translated into a functional protein that is involved in muscle force generation. However, C174T SNP association findings might be explained by LD with a functional SNP or to an influence on the stability of the RNA during the translational process.

**CNTF G-6A**

A high correlation \( r = 0.8 \) between peripheral CNTF production and muscle strength development as described in rats (11) and a highly conserved G-to-A transition in intron 1 (6 bp downstream of exon 2) producing a splice acceptor site resulting in a nonfunctional protein (31) have lead to the study of this polymorphism with muscle strength in humans (2, 27). This study tested the original hypothesis of decreased strength associated with the CNTF G-6A null allele (31). We could not find any association with this polymorphism in men. In the female middle-aged cohort, only limited evidence was present for decreased concentric KE strength at 180°/s in A/A vs. G/G homozygotes. Inconclusive results were found in the female senior cohort where G/A heterozygotes produced less isometric KE strength at 150° than both G/G and A/A homozygotes. No clear trends could be observed for any of the other strength phenotypes, which is partly due to a small number of A/A homozygotes. However, subanalyses of A-allele carriers \((n = 31/n = 21)\) vs. G/G homozygotes \((n = 106/n = 80)\) resulted in nonsignificant findings.

The findings of Roth et al. (27) pointed toward higher knee torques for G/A heterozygotes compared with both homozygote groups in a cohort of healthy men and women, which was not completely in accordance with their original hypothe-
correction for multiple testing, a conservative level was set at 0.05; however, when applying Bonferroni different study populations (Table 1). Second, significance sample size in some interaction groups, a number of interactions in all cohorts between a combination of alleles in the Research Fund of the Katholieke Universiteit Leuven (OT/04/44). M. A. I. Thomis of the Fund for Scientific Research Flanders (FWO). G. De Activity, and Health. Genotyping was supported by a Research grant to by the Flemish Government in the Policy Research Centre Sport, Physical

**Interactions of CNTF × CNTFR**

As proposed by Roth et al. (26) we tested allele × allele interactions in all cohorts between a combination of alleles in CNTFR and the CNTF null allele. However, due to small sample size in some interaction groups, a number of interactions could not be tested. Future studies should focus on larger groups to detect allele interaction effects.

Although some interesting trends were observed, cautious interpretation of the results is induced by some limitations. First, allele frequencies of the rare alleles are low for the different study populations (Table 1). Second, significance level was set at 0.05; however, when applying Bonferroni correction for multiple testing, a conservative value would need to be applied [e.g., \( P = 1 - (1 - \alpha)^{1/n} \approx 0.00029; n = 4 \) SNPs × 11 strength tests × 4 samples = 176], and none of our results would reach significance. Another limitation of this study is the estimation of FFM by anthropometric measures [Durnin-Womersley (39)], without correction for internal abdominal fat. Different regional contributions of fat (internal abdominal fat vs. subcutaneous adipose tissue) by age might confound the use of FFM as covariate between young and old subjects.

In summary, although not all of our results are in line with findings from previous studies (2, 4, 26, 27) because of different age cohorts, ethnicities, covariates, and inheritance models, we demonstrated that CNTF and CNTFR might explain interindividual variation in muscle strength phenotypes between men and women of different ages. Furthermore, our results provide evidence for sex- and age-specific effects. Although \( P \) values do not reach multiple testing-corrected thresholds, results are most consistent for the KF measures where older male T carriers of the CNTF C-1703T polymorphism perform better on all strength measurements than the C/C homozygotes. Furthermore, middle-aged female A carriers of the CNTFR T1069A polymorphism exhibit lower concentric and isometric strength than the T/T homozygotes. Given the importance of muscle strength in performing ADLs independently in the elderly and the possibility of gene therapies in the future to prevent or attenuate strength loss, research on larger sample sizes in men and women during the entire middle-aged age span is necessary.

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