Variation in the Ciliary Neurotrophic Factor Gene and Muscle Strength in Older Caucasian Women

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OBJECTIVES: To determine whether genetic variants in the ciliary neurotrophic factor (CNTF) gene are associated with muscle strength in older women.


SETTING: Twelve contiguous ZIP code areas in Baltimore, Maryland.

PARTICIPANTS: Three hundred sixty-three Caucasian, community-dwelling women aged 70 to 79.

MEASUREMENTS: Participants were genotyped at the CNTF locus for eight single nucleotide polymorphisms (SNPs), including the null allele rs1800169. The dependent variables were grip strength and the frailty syndrome, identified as presence of three or more of five frailty indicators (weakness, slowness, weight loss, low physical activity, exhaustion). In addition to genotypes, independent variables of body mass index (BMI) and osteoarthritis of the hands were included.

RESULTS: Using multivariate linear regression, single SNP analysis identified five SNPs significantly associated with grip strength ($P<.05$), after adjusting for age, BMI, and osteoarthritis. Haplotype analysis was performed, and a single haplotype associated with grip strength was identified ($P<.01$). The rs1800169 null allele fully explained the association between this haplotype and grip strength under a recessive model, with individuals homozygous for the null allele exhibiting a 3.80-kg lower (95% confidence interval $= 1.01–6.58$) grip strength. No association was seen between the CNTF null allele and frailty.

CONCLUSION: Individuals homozygous for the CNTF null allele had significantly lower grip strength but did not exhibit overt frailty. Larger prospective studies are needed to confirm this finding and extend it to additional populations. J Am Geriatr Soc 54:823–826, 2006.

Key words: muscle strength; frailty; genetics; ciliary neurotrophic factor; older women

Decline in muscle strength contributes to functional decline, disability, frailty, and falls in older adults.1–6 The molecular mechanisms that underlie these declines in strength are multifactorial, with age-related neuronal and hormonal changes, physical activity, and genetic variation all contributing to intra-individual differences. Ciliary neurotrophic factor (CNTF) is a member of the interleukin 6 (IL-6) family, with trophic effects on neuronal7 and skeletal muscular tissues.8 Sciatic nerve CNTF levels are positively associated with swimming performance and muscular strength in rats.9 In addition CNTF levels decline with age, and exogenous CNTF administration in older rats increases muscular strength.9

A G-to-A deoxyribonucleic acid sequence variant (single-nucleotide polymorphism (SNP)) was identified in the human CNTF gene that results in aberrant splicing leading to a null allele (rs1800169 A allele).10 While was not observed an association with neuromuscular disease, a subsequent study showed an association between this null allele and muscular strength in a cross-sectional case-control study of 494 individuals aged 20 to 90.11 As expected, individuals homozygous for the null allele had reduced muscular strength; however, heterozygous individuals exhibited increased muscular strength compared to individuals homozygous for either the wild-type or null alleles.

Given the importance of decreased muscle strength as a contributing factor to functional decline and frailty in older individuals and the important role that CNTF plays in muscle biology, it was hypothesized that CNTF gene variation would modulate strength decline, and the effects of genetic variants in the CNTF gene, including the null allele, on muscle strength and frailty were examined in a cross-sectional population-based cohort of older women (aged...
methods and validation results have been published previously. These analyses were performed using SAS version 8 (SAS Institute, Inc., Cary, NC).

Pairwise linkage disequilibrium estimates \( (D^2 \text{ and } r^2) \) and departures from Hardy–Weinberg proportions were estimated for all nine SNPs. Haplotypes, which define the set of SNP alleles inherited together on one chromosome, were estimated using an expectation-maximization algorithm. Subsets of SNPs to be included in a haplotype block were estimated using a confidence interval method previously developed. All descriptive haplotype calculations were performed and displayed graphically using the Haploview (V2.04) software (www.broad.mit.edu/personal/jcbarrett/haplo/index.php). The haplotype assignments were then implemented in a general linear model framework following the method of Schaid implemented in HaploStats software for R, assuming an additive model. This method estimates regression coefficients for each haplotype category, corresponding to a unit increase in the dependent variable proportional to the number of copies of a particular haplotype. Global \( P \)-values were obtained empirically, representing the overall effect of diplotype (haplotype pair) status on the dependent variable, rather than focusing on any particular haplotype a priori.

RESULTS

Eight SNPs encompassing the CNTF gene and 60 kb of surrounding sequence were genotyped in 363 older Caucasian women (aged 70–79). Genotype distributions were in agreement with Hardy–Weinberg equilibrium at the \( P < .05 \) level.
SNPs that are in close proximity to each other are often inherited as a group and are often correlated with each other at the population level. Subsets of SNP alleles inherited together over generations are often considered a haplotype “block,” and because SNPs in a block are all highly correlated, they can act as markers (surrogates) for each other. Using Haploview, a single haplotype block encompassing the CNTF gene region was identified, and four SNPs were sufficient to fully identify all the haplotype alleles (rs948562, rs1800169, rs550942, rs4319530). Haplotype analyses were performed with these SNPs. Only the haplotype containing the rs1800169 null allele was significantly associated ($P < .01$), and this SNP was sufficient to distinguish the associated haplotype from all others (Table 1), indicating that this SNP is likely the functional variant underlying the association between CNTF and grip strength. Although the null allele was not significantly associated with hip and knee strength, the direction of the effect was the same for these measures of muscle strength, and the 95% CIs overlapped with that of grip strength (data not shown). Given the strong correlation between muscle strength and frailty, multivariate linear regression analyses were performed to evaluate whether the null allele is associated with frailty, which was manifest in 35 of 363 (9.6%) study participants. No significant association was observed.

**DISCUSSION**

In this study it was determined that the rs1800169 CNTF null allele SNP accounts for the variation in grip strength identified in a comprehensive SNP and haplotype analysis of the CNTF gene. Experiments in model organisms have demonstrated that CNTF plays a role in muscle strength, and this finding was extended to a human population that ranged in age from 20 to 90. The exact role of CNTF is unclear, but a subsequent study suggested that CNTF was likely to function in the maintenance of postnatal motor neurons. Observations that CNTF null mice exhibit normal development of motor neurons during embryonic development and the first postnatal weeks but with increasing age exhibit a reduction in muscle strength support this hypothesis. These findings led to a focus on the effects of CNTF on muscle strength and frailty in older subset of the population (70–79).

The entire CNTF gene was screened using multiple SNPs across the gene and 60 kb of surrounding sequence for association with muscle strength and frailty. A positive result was found for grip strength, and based on haplotype

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**Table 1. CNTF Haplotype Analysis for Muscle Strength**

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>rs948562</th>
<th>rs1800169*</th>
<th>rs550942</th>
<th>rs4319530</th>
<th>Frequency</th>
<th>Beta&lt;sup&gt;Ⅰ&lt;/sup&gt;</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>G</td>
<td>A</td>
<td>A</td>
<td>G</td>
<td>0.169</td>
<td>−2.45</td>
<td>.01&lt;sup&gt;Ⅰ&lt;/sup&gt;</td>
</tr>
<tr>
<td>B</td>
<td>A</td>
<td>G</td>
<td>A</td>
<td>G</td>
<td>0.244</td>
<td>−0.73</td>
<td>.47</td>
</tr>
<tr>
<td>C</td>
<td>G</td>
<td>G</td>
<td>A</td>
<td>G</td>
<td>0.033</td>
<td>0.66</td>
<td>.51</td>
</tr>
<tr>
<td>D</td>
<td>A</td>
<td>G</td>
<td>A</td>
<td>A</td>
<td>0.397</td>
<td>1.25</td>
<td>.21</td>
</tr>
<tr>
<td>E</td>
<td>A</td>
<td>G</td>
<td>G</td>
<td>A</td>
<td>0.156</td>
<td>1.32</td>
<td>.19</td>
</tr>
</tbody>
</table>

* rs1800169 allele A indicates the null allele.

<sup>Ⅰ</sup> Regression coefficient estimate for a linear model including the haplotype as a predictor of muscle strength, as implemented in HaploStats.

<sup>Ⅰ</sup> Significant association.
analyses, the null allele rs1800169 could entirely explain this result. The association between the null allele and grip strength under a recessive model is in partial agreement with a previous association, because both studies found a decrease in muscle strength for individuals homozygous for the null allele. In contrast, no effect was found in heterozygous individuals in the current study. This difference may be due to the different populations, because the previous study screened men and women over a much wider age range (20–90), whereas only older women (70–79) were included in the current study. Heterozygous effects, especially the “balancing selection” model reported previously, in which heterozygous individuals are actually stronger than either homozygote, are also more likely to be subject to environmental influences, as has been observed for the hemoglobin S allele in sickle cell anemia and for the ΔF508 allele in cystic fibrosis.

Despite the effect of the null allele on muscle strength reported in the current study and the high correlation between muscle strength and frailty, an association between CNTF variants and frailty was not detected. Given the complex etiology of frailty and the likely involvement of multiple genetic pathways and environmental influences, the effect of this allele on frailty may not have been large enough to be detected in this sample. If the CNTF null allele acts upon frailty in a recessive fashion, as it does for muscle strength, it is quite possible that there were simply not enough individuals homozygous for the null allele to detect an effect on frailty (n = 16). Indeed, this sample size limitation is reflected in the imprecision of the point estimate for the effect of the null allele on grip strength in this study (95% CI = 1.01–6.58), and a much larger study will be required to narrow down this estimate. In addition, the longitudinal association between CNTF variants and incident frailty should be assessed before making any conclusions.

From these analyses, it can be concluded that women homozygous for the rs1800169 null allele have significantly lower grip strength (−3.80 kg, 95% CI = 1.01–6.58), but heterozygous women do not differ from noncarriers. Nevertheless, no effect on frailty was observed, perhaps because of the limited number of homozygous individuals in this study. These findings highlight the potential importance of this neurotrophic factor in aging-related changes in muscle strength and present a possible target for future therapeutic intervention studies.

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REFERENCES