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Interleukin-6 (IL6) Genotype Is Associated With Fat-Free Mass in Men But Not Women

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We studied the association of the G-174C promoter polymorphism in the interleukin-6 gene (IL6) with total body fat and fat-free mass (FFM) in 242 men and women (IL6 genotypes: G/G, $n = 87$; G/C, $n = 100$; C/C, $n = 55$) across the adult age span (21–92 years). In men, but not women (significant genotype by sex interactions; $p = .023$–$.048$), the C/C group exhibited significantly lower total FFM than the G/G group (54.7 ± 0.8 kg vs 57.2 ± 0.7 kg, respectively, $p = .020$), as well as significantly lower FFM of the lower limbs compared with the G/G group (18.4 ± 0.3 kg vs 19.8 ± 0.3 kg, respectively, $p = .004$). No significant genotype differences were observed in total body fat mass in either men or women. The results indicate that the IL6 G-174C polymorphism is significantly associated with FFM in men but not women.

RECENT reports have shown that sarcopenia, the loss of muscle mass and strength with advancing age, is common in older individuals and is associated with functional impairment and disability (1–3). Janssen and colleagues (1) recently reported that approximately 60% of women and 45% of men aged >60 years exhibited class I sarcopenia, equivalent to a skeletal muscle mass index below one standard deviation from the mean values of young adults.

Although the etiology of sarcopenia is likely multifactorial (4), recent reports have linked interleukin-6 (IL-6) and other inflammatory cytokines to lower muscle mass and strength. For example, higher levels of IL-6 have been associated with skeletal muscle wasting in a number of animal model studies (5–7), and an IL-6 receptor antibody prevents muscle-wasting in transgenic mice with high IL-6 levels (5,8). Most recently, Visser and colleagues (9) reported that higher plasma concentrations of IL-6 were associated with lower muscle mass and strength in healthy older individuals. Thus, variation in IL-6 levels appears to be associated with differences in fat-free mass (FFM).

Several reports have described the role of a promoter polymorphism (G-174C) in the interleukin-6 gene (IL6) on IL-6 production in vitro (10,11), as well as in vivo (10,12); however, controversy exists over the influence of sex and age on any relationship (12–14). The purpose of the present study was to determine if the IL6 G-174C promoter polymorphism was associated with body weight, fat mass, nonosseous FFM (i.e., soft tissue FFM), and muscle strength in men and women across the adult age span.

Methods

Participants

Healthy Caucasian volunteers (110 men and 132 women; aged 21–92 years) from the Baltimore Longitudinal Study of Aging (BLSA) participated in the study. All participants received a complete medical history and physical examination, and those with clinical cardiovascular or musculoskeletal disorders known to be adversely affected by exercise testing were excluded. Detailed exclusion criteria are outlined elsewhere (15,16). Physical activity levels were determined from the self-reported amount of time spent in 97 activities since the last biennial visit, with energy expenditure estimated in metabolic equivalents (METS) as previously described (17). The results of a physical activity questionnaire indicated that only a small proportion of the subjects (<1%) participated in regular resistive exercise training, with no differences in participation by age, sex, or IL6 genotype (15). Menopausal status of the women in the study was not accounted for, though there were no age-by-sex interactions within any of our analyses. Experimental protocols were approved by the Institutional Review Boards at Johns Hopkins Bayview Medical Center, the University of Pittsburgh, and the University of Maryland, and all participants gave their written informed consent.

Body Composition

Body mass and height were measured for each participant to the nearest 0.1 kg and 0.5 cm, respectively, and body mass index (BMI) was calculated (kg/m^2). All participants were also assessed for total body and lower limb fat mass and FFM. A total body scan was performed using dual-energy X-ray absorptiometry (DEXA) as described previously (15,16). Based on the total body DEXA scan, total body fat and nonosseous (i.e., soft tissue) FFM were determined. FFM measured by DEXA correlates strongly with muscle mass in humans across the age span (18,19).

Muscle Strength Assessment

Peak torque (strength) was measured using the Kinetic Communicator isokinetic dynamometer (Kin-Com model

Table 1. Subject Characteristics

	Men			Women		
IL6 Genotype	G/G	G/C	C/C	G/G	G/C	C/C
N	36	47	27	51	53	28
Age (y)	54.0 ± 2.2	53.6 ± 1.9	50.8 ± 2.5	47.9 ± 1.9	50.2 ± 1.8	47.9 ± 2.5
Height (cm)	178 ± 1	178 ± 1	177 ± 1	164 ± 1	166 ± 1	163 ± 1
Weight (kg)*	81.8 ± 2.0	77.4 ± 1.8	79.9 ± 2.3	72.8 ± 1.7	73.5 ± 1.6	74.5 ± 2.3
BMI	27.8 ± 0.7	26.4 ± 0.6	27.2 ± 0.8	24.9 ± 0.5	25.2 ± 0.5	25.6 ± 0.7
Fat (kg)*	22.0 ± 1.7	20.6 ± 1.5	23.4 ± 1.9	29.0 ± 1.9	27.9 ± 1.4	28.0 ± 1.5
Activity (MET-min/24 hr)	2272 ± 66	2292 ± 56	2199 ± 75	2399 ± 55	2352 ± 53	2426 ± 74
Con30 (Nm)*	190.6 ± 7.5	178.9 ± 6.3	192.2 ± 6.8	128.6 ± 5.6	132.1 ± 5.4	126.2 ± 7.5
Con180 (Nm)*	121.6 ± 4.7	119.3 ± 3.8	125.5 ± 4.2	81.7 ± 3.4	84.2 ± 3.4	80.9 ± 4.6

Notes: Data are least-squares means ± *SE* (Standard Error).

*Means adjusted for age, height and physical activity. No significant IL6 genotype differences were observed.

Con30 = quadriceps concentric peak torque at 30°; Con180 = quadriceps concentric peak torque at 180°. MET = metabolic equivalent; BMI = body mass index; IL6 = interleukin-6.

125E, Chattanooga Group, Chattanooga, TN). Concentric peak torque was measured at angular velocities of 0.52 rad/s (30°/s) and 3.14 rad/s (180°/s) for the dominant knee extensors. For each test, participants performed three maximal efforts, separated by 30-second rest intervals, from which the highest value of the three trials was accepted as the peak torque. Detailed procedures regarding participant positioning and stabilization, warm-up, testing order, gravity correction, and Kin-Com calibration are described elsewhere (15,16). Correlation coefficients for test–retest reliability ranged from 0.96 to 0.99 (15).

Genotype

Genomic DNA was extracted from whole blood or cell lines derived from transformed lymphocytes using standard techniques. Variation at the IL6 G-174C promoter polymorphism (genotype groups: G/G, G/C, or C/C) was identified using fluorescence polarization (FP) (20) using the following primers: polymerase chain reaction (PCR) forward: 5′-tgacttcagctttactctttgt; PCR reverse: 5′-ctgattggaaaccttattaag; FP: 5′-tgtgcaatgtgacgtcctttagcat. Direct DNA sequencing was used to confirm genotypes for a random selection of samples.

Statistics

Chi-square analysis was used to determine deviation of genotype frequencies from the expected Hardy Weinberg equilibrium. Participants were grouped for the three IL6 genotypes and analyzed by analysis of variance methods with age and sex as independent variables. Weight, BMI, body fat, total FFM and lower limb FFM, and muscle strength were response variables in analysis of covariance, with physical activity, and/or height, and/or weight as covariates. Similar analyses were performed with participants grouped for presence and absence of the C allele (C/C + C/G vs G/G). Data are reported as adjusted least squares means ± *SE* (standard error), with statistical significance accepted at $p \leq .05$.

Results

A total of 242 Caucasian men and women were genotyped for the G-174C polymorphism in IL6, with 87 G/G genotypes (36.0%), 100 G/C genotypes (41.3%), and 55 C/C genotypes (22.7%). The G and C allele frequencies were 57% and 43%, respectively. Although the genotype frequencies deviated from the Hardy-Weinberg equilibrium in women (men: $p = .140$; women: $p = .050$), the allele frequencies are similar to those reported previously for European Caucasians (10).

Participant characteristics are outlined in Table 1. No significant differences were observed in the entire cohort for weight, BMI, body fat, or knee extensor strength in relation to IL6 genotype (Table 1). A significant sex-by-IL6 genotype interaction was observed for total FFM ($p = .048$), such as that in men but not women, G/G men had significantly higher FFM than both G/C and C/C men (Figure 1). An analysis of lower limb (both legs) FFM showed similar results, with a significant sex-by-genotype interaction ($p = .004$), such that G/G men, but not women, exhibited higher lower-limb FFM than both G/C and C/C men (Figure 1). We then performed an analysis comparing carriers of the C allele (G/C + C/C groups) to G/G homozygotes for total FFM and lower limb FFM, and observed similar significant sex-by-genotype interactions for both FFM measures ($p = .013$ and .006, respectively; data not shown).

Discussion

The results of the present study provide the first evidence of an association between the IL6 G-174C promoter polymorphism and soft tissue FFM. The findings indicate a sex-specific association, such that men, but not women, who are homozygous for the G allele exhibit significantly higher total and lower limb FFM than men with one or more C alleles. The G-174C polymorphism was not associated with weight, BMI, body fat, or muscle strength in either men or women. We cannot explain the lack of an association between IL6 genotype and muscle strength given the significant association with lean mass in men. In our analyses, we examined the relationship between IL6 genotype and muscle strength with and without accounting for lean mass (i.e., muscle mass), and the results consistently showed no relationship to strength. However, muscle strength can be measured in several ways, and we did not find an association with the specific measurements available to us.

The present results are consistent with recent in vitro and in vivo findings reported by Olivieri and colleagues (12) on the role of IL6 genotype on IL-6 production, with IL6 genotype influencing IL-6 production in men only (12). Others have also reported no influence of IL6 genotype on IL-6 levels in women (13). Unfortunately, IL-6 levels were not available for study in our participants, thus preventing a complete analysis of the relationship between IL6 genotype, sex, and FFM.

In addition to the uncertainty surrounding the environmental contributors of age and sex, other functional sequence variants exist in the IL6 promoter that may also influence IL-6 production. For example, Terry and colleagues (11) have reported that upstream promoter polymorphisms, especially the A_n-373T_n repeat site, also contribute to variation in in vitro IL-6 production. A significantly larger cohort than that studied here would be required to determine the role of various combinations of these polymorphisms in the IL6 promoter region on FFM. We chose to investigate the G-174C polymorphism as it has been the most studied of the IL6 promoter polymorphisms, and has been associated with insulin sensitivity, lipid abnormalities, and bone mineral density (21–24). The findings reported here will thus require verification in a large independent cohort before definitive conclusions can be made.

Our observed genotype frequencies deviated from the expected Hardy Weinberg equilibrium in women ($p = .050$), though we confirmed the genotypes through direct DNA sequencing. Thus population stratification within our all-Caucasian sample, which was primarily recruited from the mid-Atlantic region of the United States, must also be considered in light of the results, but, with the exception of exclusion criteria (see below), we can discern no obvious basis for such stratification based on the subject characteristics.

An important issue with any study that relies on exclusion criteria based on health status is the possibility of genotype-related influences on health that lead to bias in the participants included for investigation. Given the importance of inflammatory cytokines, including IL-6, in several age-associated diseases (25), we sought to address this issue of bias in the present study. We compared the 242 participants included in the present analysis to the remaining Caucasian participants who were not studied and found few significant differences in physical characteristics. For example, no differences were found between the two groups in height, weight, BMI, hip circumference, fat mass, or lean mass when adjusting for age, sex, and age by sex. Waist circumference was approximately 1 cm less in participants included in the study, and the waist:hip ratio was lower by 0.014 when adjusted for age, sex, and age by sex. In addition, there was no difference between groups in the prevalence of diabetes or myocardial infarctions. Angina was more frequent in the participants who were not included (prevalence 2.3%). Overall, participants who were evaluated in the present study did not differ greatly from those participants who were not studied, and differences, when present, between the two groups were small.

The findings reported here represent some of the first to identify a specific gene variant associated with FFM in humans (26–29), which might have future importance for identifying individuals at risk for sarcopenia. Considerable work will be required to fully identify the specific genes that contribute to FFM, and more specifically that contribute to sarcopenia.

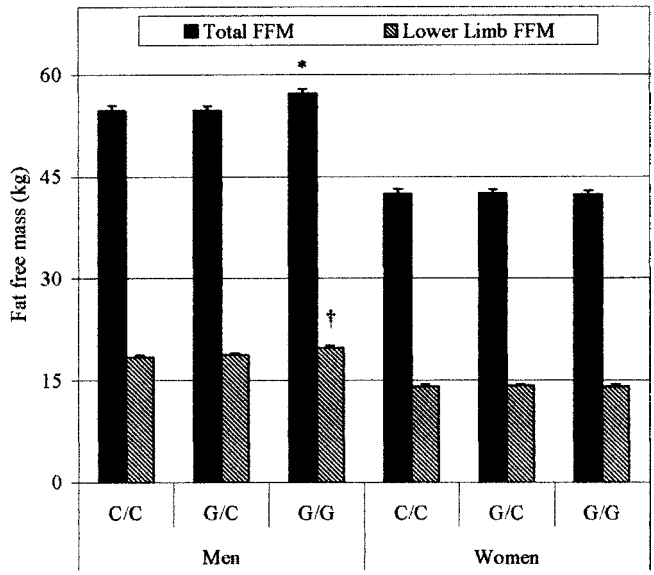

Figure 1. Total nonosseous (soft tissue) fat-free mass (FFM) and lower limb FFM are shown as least squares means ± *SE* (standard error) adjusted for age, height, and physical activity for men and women grouped by IL6 genotype (C/C, G/C, and G/G). *$p = .01$ and .02 versus G/C and C/C group for men, respectively. †$p = .01$ and .004 versus G/C and C/C group for men, respectively. Significant sex-by-IL6 genotype interactions were observed for both total FFM ($p = .048$) and lower limb FFM ($p = .023$).

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