Polymorphic Variation in the Human Myostatin (GDF-8) Gene and Association with Strength Measures in the Women’s Health and Aging Study II Cohort

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OBJECTIVES: To determine whether polymorphic variation in the myostatin gene differentially influences the maintenance of muscle strength in older adults, and to find supportive evidence in a cohort of older women.

DESIGN: Correlation study of polymorphic variation in a cohort of older women.

SETTING: Representatively sampled older female population living in the eastern half of Baltimore, Maryland.

PARTICIPANTS: Participants were 286 women, age 70 to 79. Of these, 81.1% were Caucasian, 18.8% were African-American, and 0.2% were Asian or Hispanic.

MEASUREMENTS: Overall strength was measured with a dynamometer and defined as the sum of the strongest measures of hip, knee, and grip strength on the dominant side.

RESULTS: We identified or confirmed six myostatin polymorphic variants in the Women’s Health and Aging Study II population. Of the polymorphisms, K153R is the most common, with an allele frequency of 0.19 in African Americans. Unadjusted mean strength by genotype suggested lower muscle strength in those African-American women with the R genotype compared with those with the K genotype (K/K: 72.50 ± 13.9 kg (n = 39) vs K/R: 67.14 ± 11.4 kg (n = 13) vs R/R: 63.1 ± 11.3 kg (n = 3)). After adjustment for race in a linear regression model, the R genotype remained associated with lower strength levels (P = .04). Statistical significance decreased when body mass index and race were both added to the model (P = .09).

CONCLUSIONS: Recognizing that small sample size in the study of genes of modest effect are unlikely to yield significant differences, these data suggest an association of the R153 allele with lower strength in high-functioning older women, which should be studied further in a larger cohort. J Am Geriatr Soc 49:1093–1096, 2001.

Key words: sarcopenia; skeletal muscle; polymorphism; myostatin; aging

SK: Skeletal muscle mass declines with age (sarcopenia), and this loss markedly influences metabolism, mobility, and strength. The molecular mechanisms that underlie lean body mass decline are complex but are thought to be secondary to age-related alterations in neuronal and hormonal backgrounds and environmental and genetic variability. Myostatin, or growth-differentiation factor-8 (GDF-8), is a member of the transforming growth-factor-β (TGF-β) superfamily of related proteins and has been shown to be a strong negative regulator of muscle mass in murine models. Myostatin null mice showed a two- to threefold increase in skeletal muscle mass, and Piedmontese and Belgian Blue cattle were found to have missense mutations in the myostatin coding sequence that leads to skeletal muscle hypertrophy and hyperplasia. In humans, a study of human immunodeficiency virus-positive men demonstrated a strong association of increased levels of serum myostatin and muscle wasting. Ferrell et al. recently identified five human polymorphisms in the myostatin gene and studied the two most common polymorphisms for association with differential muscle mass response to strength training in Caucasian (n = 107) and African-American (n = 41) subjects. Although no significant gene impact on muscle mass response to strength training in either ethnic group was detected, one polymorphism (R153) was overrepresented in the nonresponder group, suggesting that this allele may play a role in other muscle phenotypes.

Given the important functional role of myostatin in the maintenance of muscle mass in rodents, naturally occurring gene polymorphisms that predict increased muscle mass in cattle, associations between high serum myostatin levels and muscle wasting in humans, and newly identified human polymorphisms in the myostatin gene that suggested a differential in muscle development in response to
exercise, we hypothesized that variation in the human myostatin gene may play a role in age-related changes in muscle mass and muscle strength. Identification of clinically relevant gene variants or gene regulatory sequences may further the development of targeted interventions for the prevention or treatment of sarcopenia.

METHODS

Human Subjects

Subjects were recruited from the Women’s Health and Aging Study II (WHAS II), a prospective, observational study of physical function in a cohort selected to be representative of the highest-functioning two-thirds of women age 70 to 79 living in the eastern half of Baltimore, Maryland.11 The Johns Hopkins Institutional Review Board approved the use of human subjects. Four hundred thirty-six women were enrolled in the study, from a sample drawn from the Health Care Financing Administration Medicare eligibility lists. Full description of sampling and recruitment methodology has been published.12 Of these women, 18.8% were African American, 0.2% were Asian or Hispanic, and 81.1% were Caucasian. Of these 436 women, 286 consented for genotyping and were included in this analysis. From these 286 participants, we first randomly chose a representative subset of the 25 strongest and 25 weakest for screening to maximize our ability to detect functionally significant myostatin gene variants. The racial makeup of this subset was the same as the overall WHAS II cohort.

Study participants were assessed at a baseline clinical examination at Johns Hopkins Hospital General Clinical Research Center and at two subsequent visits 18 months apart. Although no direct measurement of muscle mass is available, measurements of grip, hip, and knee strength are available for all participants. For grip strength measurements, a JAMAR hand dynamometer (model BK-7498, Fred Sammons, Inc., Burr Ridge, IL) was used. Grip strength was measured three times with the dominant hand and the strongest reading was used for our analysis. Knee extension strength was measured in two trials on the left lower extremity and in two trials on the right lower extremity using a hand-held isometric dynamometer (model 01160, Lafayette Instruments, Lafayette, IN). The strongest measurements from each side were averaged. Hip flexion strength was also an average of the strongest measurement from each side and was measured with the same dynamometer. The overall strength measure is the sum of the strongest measures of hip, knee, and grip strength.

Single-Stranded Conformational Polymorphism Analysis and Genotyping

We first screened the subset of the 25 strongest and 25 weakest WHAS II participants for myostatin gene variants using single-stranded conformational polymorphism (SSCP) analysis.13 Primers selected from genomic sequence were used to screen the 5′ untranslated region (UTR) and all three exons. Standard polymerase-chain reaction conditions were used with the additional supplement of 2 μCi of [α-32P]-dCTP for radiolabelling. All polymerase-chain reaction products were denatured at 95°C for 5 minutes and electrophoresed on a Mutation Detection Enhancement gel (FMC Bioproducts, Rockland, ME) with or without glyceral at 4°C or 25°C. Gels were vacuum/heat dried and exposed to autoradiograph film at −70°C overnight.

We then genotyped the consented participants in the WHAS II cohort for identified polymorphisms using polymerase-chain reaction–restriction fragment length polymorphism analysis and enzymes specific for each polymorphism (see results). One newly identified missense mutation in exon 1, which lacked a common restriction site, was not genotyped in this cohort. Digested products were resolved on either 3% or 4% agarose gels containing ethidium bromide and visualized under ultraviolet light.

RESULTS

We identified six polymorphic variants within the myostatin gene in the 25 strongest and 25 weakest participants using SSCP. Of these six, three were newly identified polymorphisms and three were confirmatory of previously identified polymorphisms.10 We identified two new polymorphisms within the 5′ UTR, including a 4 base pair deletion, designated Dde I; a C to A substitution, designated Nla III; and one new missense substitution in the first exon (R65H). We were unable to identify a common restriction site for this variant. Additionally, we confirmed three missense substitutions: one in the first exon (A55T, digests with HaeIII) and two in the second exon (K153R, digests with ApaI and I225T, digests with Stu I). No polymorphic variants were identified in exon 3. After genotyping the entire group of 286, we found that the variant genotypes were very uncommon in Caucasian populations, with a gene frequency less than 0.02 for all polymorphisms. A higher allele frequency for three variant alleles was noted in the African-American participants from WHAS II, with a gene frequency of 0.05 for the Nla III polymorphism, 0.07 for the Hae III polymorphism, and 0.17 for the Apa I polymorphism.

Because of this higher allele frequency, we were able to perform association studies between this variant allele (K153R or ApaI polymorphism) and strength measurements. Unadjusted mean strength by K153R genotype suggested lower muscle strength in those women with the R genotype (Table 1). We therefore evaluated whether this allele variant was independently associated with strength in these high-functioning older women. Because of the small number of homozygotes for the R allele, a limited ability to detect statistical significance in such a small group, and evidence from a previous publication that one or two copies of the R allele may be related to low response to training, we combined the heterozygous (K/R) and homozygous (R/R) groups for analytical purposes.10 We then compared that group to those with the K/K genotype as predictors of strength in multiple linear regression analyses, adjusting for race and body mass index (BMI) (Table 2). Both race and BMI have been demonstrated to affect muscle strength significantly. Genotype was a significant predictor of overall strength when adjusting for race. The significance decreased to borderline when the model was additionally adjusted for BMI in this sample of 50 women. However, the slope estimate of −5.04 indicates that the overall strength for those with either one or two copies of the R allele was 5.04 kg lower than that for the K/K group, after controlling for race and BMI (P < .09). Additionally, these analyses indicate that, for two
persons with the same genotype and same BMI, and in the same 10-year age group, African Americans tended to have greater overall strength, about 6.3 kg higher than Caucasians ($P < .01$). We additionally tested for an interaction between race and genotype, but this was not significant (data not shown). Therefore, there was insufficient evidence to support the hypothesis that there is a differential racial effect on the association between this genotype and overall strength.

Because of recent evidence that indicates myostatin is differentially expressed in muscle groups, we also evaluated the relationship of the variant allele to hip, knee, and grip strength in addition to the overall strength measurement, using the same linear regression model (Table 2). We found that most of the genotype difference was generated by hip flexion strength differences. The slope of the estimate of $-4.00$ indicates that, for those individuals with the variant allele, hip flexion was 4 kg lower than in those without the variant allele after adjustment for race and BMI ($P < .01$). Knee extension strength and grip strength was not significantly different between genotypes.

We also evaluated mean overall strength in the three other genotypes. For the N1a III polymorphism, mean strength ± standard deviation for cytosine/cytosine genotype in African Americans was $71.10 ± 13.45$ ($n = 48$) and for cytosine/adenine $70.49 ± 12.46$ ($n = 5$). We identified no Caucasians with this variant. For the Hae III variant, the mean strength for Ala/Ala for the African-American group was $70.55 ± 13.28$ ($n = 46$) versus $68.59 ± 11.93$ ($n = 8$) in the Ala/Thr group. We identified only one Caucasian with this variant. For the Str I variant, we identified no African Americans with the variant and four Caucasians. For Caucasians, mean strength of the Ile/Ile group was $62.90 ± 12.51$ ($n = 228$) versus $69.57 ± 10.47$ ($n = 4$). Because of limited power to detect statistically significant differences, we did not perform linear regression analysis with these genotypes.

**DISCUSSION**

We have identified or confirmed several human myostatin genetic variants in the WHAS II population and describe here the relationship between the most common variant and strength in this cohort of aging women. Given the modest effect that any single gene would be expected to have on muscle strength and the level of association observed here, the K153R variant warrants further investigation into its role in differential strength decline and myostatin levels in older adults. The WHAS II population consists of a representative sample of the two-thirds least-disabled women age 70 to 79 with generally high mobility function. If the R genotype is associated with lower strength, as suggested in this study, the R genotype may actually be underrepresented here because the more-disabled women age 70 to 79 were not recruited for the WHAS study. Because older women are at higher risk of functional decline related to sarcopenia than are older men, studies of larger cohorts representative of the entire population of older women or, particularly, of a more representative African-American cohort would enhance the ability to define the allelic effect of this polymorphism on strength.

We have also identified a significant difference in hip flexion strength by K153R myostatin genotype that explains most of the difference in the combined strength measure. The major hip flexor muscle is the ilopsoas muscle, which is primarily composed of type 1 muscle fibers. Myo-
Statin is expressed at higher levels in type 1 muscle fibers and may therefore have a more significant functional impact in this muscle group. Further functional analysis of this gene variant and expression differences in predominantly type 1 muscle group remains to be performed.

In this cohort, which was used to study functional decline in older women, we were not able to address the relationship between the myostatin gene variant and muscle mass. To date, most studies have evaluated the relationship between myostatin and muscle mass rather than the relationship between myostatin and muscle strength. It is important to note that muscle mass in humans does not always correlate directly with muscle strength, and that muscle strength loss cannot be uniformly explained by changes in muscle size. Therefore, confirmatory studies evaluating both serum myostatin levels and muscle mass in relationship to this genotype and to muscle strength are needed to help clarify these important questions.

REFERENCES