Androgen receptor CAG repeat polymorphism is associated with fat-free mass in men

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Walsh, Sean, Joseph M. Zmuda, Jane A. Cauley, Patrick R. Shea, E. Jeffrey Metter, Ben F. Hurley, Robert E. Ferrell, and Stephen M. Roth. Androgen receptor CAG repeat polymorphism is associated with fat-free mass in men. J Appl Physiol 98: 132–137, 2005. First published September 17, 2004; doi:10.1152/japplphysiol.00537.2004.—The human androgen receptor (AR) gene contains a CAG (glutamine) repeat polymorphism in exon 1 that is inversely associated with transcriptional activity of the AR. We studied the association of AR CAG repeat length, fat-free mass (FFM), and testosterone in two independent cohorts: 294 Caucasian men, aged 55–93 yr, from the Study of Osteoporotic Risk in Men (STORM), and 202 Caucasian volunteers (112 men and 90 women), aged 19–90 yr, from the Baltimore Longitudinal Study of Aging (BLSA). Subjects were genotyped to determine the number of AR CAG repeats and grouped as carrying either <22 or ≥22 repeats. Whole body soft tissue composition was measured by dual-energy X-ray absorptiometry. Men with greater CAG repeat number exhibited significantly greater total FFM than those with fewer CAG repeats in both cohorts (STORM: 59.2 ± 0.3 vs. 58.0 ± 0.4 kg, P = 0.02; BLSA: 57.2 ± 1.1 vs. 54.3 ± 1.1 kg, P = 0.04). Similar results were observed for total FFM normalized to height. No differences were seen in women in the BLSA cohort. In the BLSA cohort, serum testosterone levels were higher in subjects with greater repeat number (P = 0.003). This same pattern approached significance in the STORM cohort (P = 0.07). In conclusion, the androgen receptor CAG repeat polymorphism is associated with FFM in men in two independent cohorts. Additional studies are needed to confirm this observation and to clarify the mechanisms involved.

MUSCLE MASS AND STRENGTH are highly heritable phenotypes, and genetic factors contribute significantly to the variation in lean body mass and muscle strength (1, 5, 42). To date, information identifying genes and their allelic variants that contribute to muscle-related phenotypes is limited to the vitamin D receptor (12, 36, 44, 45), ciliary neurotrophic factor and its receptor (33, 34), myostatin (8, 18, 37), insulin-like growth factor I (40), type I collagen (46), and interleukin-6 (35).

Androgen receptors (AR) are highly expressed in skeletal muscle (4, 38), with expression being upregulated in response to muscle overload (2). Animal and clinical studies have indicated that the androgen-AR signaling pathway is required for both skeletal muscle development and increases in muscle mass, strength, and muscle protein synthesis in response to androgens (24). The AR is a member of the nuclear receptor family of ligand-activated transcription factors and is divided into three functional domains (27): the NH2-terminal domain is important for transcriptional activation of androgen-responsive genes (19), the central region encodes a DNA-binding domain, and the COOH-terminal portion contains the ligand-binding domain (14).

The AR gene contains a polymorphic CAG microsatellite repeat sequence, which normally ranges from 8 to 31 repeats and averages ~20 repeats in length (11). The CAG repeat is present in exon 1 and encodes for a polyglutamine chain in the NH2-terminal transcriptional activation domain of the AR gene. Several reports indicate that longer CAG repeat length in the human AR results in a linear decrease of transactivation function (6, 7, 20, 43).

Previous studies have shown associations of the AR CAG repeat polymorphism with androgen-related conditions, such as benign prostatic hypertrophy, prostate cancer, and male infertility (13, 23, 30). Given the importance of androgens to the development and maintenance of muscle mass, the importance of the CAG repeat polymorphism to AR function, and the known heritability of muscle mass, we hypothesized that individuals with a greater number of AR gene CAG repeats would exhibit lower levels of total fat-free mass (FFM) compared with individuals with fewer CAG repeats. We tested our hypothesis in two independent cohorts derived from the Study of Osteoporotic Risk in Men (STORM) and the Baltimore Longitudinal Study of Aging (BLSA).

METHODS

Subjects. The STORM cohort consisted of a sample of 294 Caucasian men, aged 55–93 yr, recruited primarily from population-based listings (15). Men who were unable to walk without the assistance of another person or had undergone a bilateral hip replacement were ineligible to participate in STORM.

The BLSA cohort consisted of 202 Caucasian volunteers (112 men and 90 women), aged 19–90 yr, recruited primarily from population-based listings. Details of the BLSA recruitment methodology are outlined elsewhere (39). Additional BLSA men without body composition data were included in the analysis of testosterone levels (total n = 163). All BLSA subjects received a complete medical history and physical examination, and subjects with clinical cardiovascular or musculoskeletal disorders that could be adversely affected by exercise testing were excluded.

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All subjects gave their written, informed consent before participation and received a complete medical history and physical examination. The experimental protocols were approved by the Institutional Review Boards for Human Subjects at Johns Hopkins Bayview Medical Center (Baltimore, MD) and at the University of Pittsburgh (PA). The protocols related to the analysis of genetic data were approved by Institutional Review Boards at the University of Maryland (College Park, MD) and the University of Pittsburgh.

AR CAG repeat polymorphism. DNA was isolated from EDTA anticoagulated whole blood by using standard methods. PCR amplification of the AR CAG repeat was performed by using forward primer ARA-F 5' -ACCGGAGGACCTCAGGAT - 3' and reverse primer R1 5' - AGAACCATTCTACACCCGTCT - 3'. Fluorescently labeled with HEX or FAM and one of the following reverse primers: ARA-R1 5' - AGAACCATTCTACACCCGTCT - 3' and ARA-R3 5' - AAGCTGAGTACCTCTCGTCCC- 3'. The use of three reverse primers allowed for three samples to be assayed simultaneously yet unambiguously, thus improving efficiency. For example, three samples each with 21 CAG repeats would yield fragment sizes of 230, 296, and 420 bp, respectively. PCR products for each of the amplicon sizes and dye colors (6 total fragments) were combined per lane. PAGE analysis was then performed by using an ABI 377 automated sequencer. An internal control of known repeat number was included with each amplification.

Whole body soft tissue composition was measured by dual-energy X-ray absorptiometry, using previously described methods (26). Appendicular muscle mass was considered equivalent to the sum of the muscle masses of the right and left limbs, using a medical beam scale, and body mass index was calculated for each subject to the nearest 0.1 kg and 0.5 cm, respectively. PCR products for each of the amplicon sizes and dye colors (6 total fragments) were combined per lane. PAGE analysis was then performed by using an ABI 3700 automated sequencer. Once allele length was determined; it was converted to CAG repeat number via the following formula: (allele size - 221)/3. The median allele length of 287 is equal to 22 CAG repeats.

Body composition. Volunteers from the STORM cohort had their body weight measured to the nearest 0.1 kg on a calibrated balance beam scale. Height was measured to the nearest 0.1 cm after removal of shoes, at the peak of inhalation, using a wall-mounted Harpenden stadiometer (Holtain, Dyved, UK). The average of two height measurements was recorded, and body mass index was calculated (kg/m²).

Muscle strength testing. For the STORM cohort, grip and knee extensor strength were measured by using the Bodystar Isometric Dynamometer (Dublin, CA) and Jackson Evaluation System (Lafayette Instrument), as previously described (36). Participants had one practice trial and two trials on each leg. Each trial consisted of 4 s of contraction, with force measurements recorded during the last 3 s. For the BLSA cohort, peak torque (strength) was measured by using the Kinematic Communicator isokinetic dynamometer (Kin-Com model 125E, Chattanooga Group, Chattanooga, TN). Concentric and eccentric peak torque were 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, subjects performed three maximal efforts, separated by 30-s rest intervals, from which the highest value of the three trials was accepted as the peak torque. Detailed procedures regarding subject positioning and stabilization, warm-up, testing order, gravity correction, and Kin-Com calibration are described elsewhere (26, 28).

Testosterone. For the STORM cohort, blood samples were obtained in the morning between 0700 and 1000, after an overnight fast. Samples were stored at −70°C. Total testosterone was measured by RIA after extraction and purification by LH-20 column chromatography. Bioavailable testosterone was determined by ammonium sulfate precipitation process that separates the sex hormone-binding globulin (SHBG)-bound steroid and free steroid. Serum samples were incubated in [3H]testosterone. SHBG was precipitated by the addition of ammonium sulfate at a concentration of 50% saturated. The samples were centrifuged, and aliquots of the supernatant containing the "non-SHBG-bound" steroid were used for scintillation counting. The bioavailable testosterone concentration was then derived from the product of the total serum testosterone and the percent non-SHBG-bound steroid determined from the separation procedure.

For the BLSA cohort, blood samples were obtained in the morning between 0700 and 0930, after an overnight fast. Before 1992, samples were stored at −20°C. Samples collected after 1992 were kept at −80°C. Total testosterone levels were determined, in duplicate, by using 125I-doubled-antibody RIA kits obtained from Diagnostic Systems Laboratories (Webster, TX). Complete details are described elsewhere (17).

Physical activity. STORM participants completed a self-administered questionnaire, which was reviewed with each participant in the clinic by a trained interviewer. Physical activity was measured by using a modified Paffenbarger scale (31) in which subjects reported the frequency and duration of their participation per week during the past year in 33 different physical activities. The activities were assigned energy expenditures, according to previously reported methods (32), and total physical activity, expressed in kilocalories expended per day, was calculated by summing the kilocalories expended in the 33 recreational activities.

Physical activity was estimated for each of the BLSA participants using self-reported time spent in 97 activities, as has been previously reported (41, 48). Physical activity was quantified into metabolic equivalent-minutes based on the metabolic equivalent of each particular activity and the time spent in that activity, normalized to 24 h.

Statistics. Subjects were grouped according to the number of AR CAG repeats, based on the median value in both cohorts, which resulted in the most balanced grouping. Thus individuals were grouped as harboring repeat lengths of <22 (i.e., short allele) or ≥22 CAG repeats (i.e., long allele). Only females homozygous for a short allele or homozygous for a long allele at the X chromosome locus were included in the analysis; females carrying both a short allele and a long allele were excluded.

Subject characteristics were analyzed by using t-tests between the two repeat-length groups. Dependent variables (FFM and testosterone variables by AR CAG repeat number in 294 adult men from the STORM cohort

<table>
<thead>
<tr>
<th>n</th>
<th>≥22</th>
<th>P Value</th>
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<tbody>
<tr>
<td>n</td>
<td>120</td>
<td>174</td>
</tr>
<tr>
<td>Age, yr</td>
<td>72.3±0.7</td>
<td>73.5±0.5</td>
</tr>
<tr>
<td>Height, cm</td>
<td>173±0.6</td>
<td>173.5±0.5</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>83.6±1.2</td>
<td>83.9±1.0</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.8±0.4</td>
<td>28.1±0.3</td>
</tr>
<tr>
<td>Physical activity, kcal/day</td>
<td>400±22</td>
<td>400±18</td>
</tr>
<tr>
<td>Appendicular muscle mass, kg</td>
<td>23.8±0.2</td>
<td>24.1±0.2</td>
</tr>
<tr>
<td>Relative appendicular muscle mass, kg/m²</td>
<td>7.9±0.1</td>
<td>8.0±0.1</td>
</tr>
<tr>
<td>Total FFM, kg</td>
<td>58.0±0.4</td>
<td>59.2±0.3</td>
</tr>
<tr>
<td>Relative total FFM, kg/m²</td>
<td>19.4±0.1</td>
<td>19.8±0.1</td>
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Values are least squares means ± SE; n, no. of subjects. BMI, body mass index; FFM, fat-free mass; AR, androgen receptor; STORM, Study of Osteoporotic Risk in Men.
Table 2. Muscle strength values by AR CAG repeat number in the STORM cohort

<table>
<thead>
<tr>
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<th>&lt;22</th>
<th>≥22</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average quadriceps strength</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted for FFM*</td>
<td>40.0±1.3</td>
<td>41.1±1.1</td>
<td>0.52</td>
</tr>
<tr>
<td>Adjusted for FFM†</td>
<td>36.7±1.3</td>
<td>36.5±1.1</td>
<td>0.85</td>
</tr>
<tr>
<td>Peak quadriceps strength</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted for FFM*</td>
<td>36.4±1.4</td>
<td>36.6±1.2</td>
<td>0.91</td>
</tr>
<tr>
<td>Adjusted for FFM†</td>
<td>40.4±1.3</td>
<td>40.8±1.1</td>
<td>0.79</td>
</tr>
<tr>
<td>Grip strength</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted for FFM*</td>
<td>30.8±0.5</td>
<td>31.8±0.4</td>
<td>0.13</td>
</tr>
<tr>
<td>Adjusted for FFM†</td>
<td>30.7±0.5</td>
<td>31.8±0.4</td>
<td>0.13</td>
</tr>
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</table>

Values are least squares means ± SE in kg. *All analyses were corrected for age, height, fat mass, and physical activity. †In addition to the other covariates, the model was adjusted for appendicular FFM. There were no significant differences between repeat groups.

variables) were analyzed by using analysis of covariance for both cohorts. For the STORM cohort, age, body fat mass, height, and physical activity were significant covariates for the analysis of FFM variables, with appendicular FFM used as an additional covariate for the analysis of muscle strength. Multivariate regression models were also performed in the STORM cohort to test for the association of AR CAG repeat number as a continuous variable. In addition, analyses were performed by using approximate tertiles of repeat number for the analysis of testosterone data in the STORM cohort. For the BLSA cohort, AR repeat length and sex were used as independent variables, analysis of muscle strength. Multivariate regression models were performed by using approximate tertiles of repeat number for the analysis of testosterone data in the STORM cohort. For the BLSA cohort, AR repeat length and sex were used as independent variables, analysis of muscle length of 0.8% for both total FFM and relative total FFM. No significant differences were observed for muscle strength values between AR repeat groups (Table 2).

RESULTS

STORM cohort. Subject characteristics and soft tissue FFM variables grouped by AR CAG repeat number in 202 Caucasian adult men and women from the BLSA cohort are shown in Table 3, grouped by median AR repeat length, which was 22 repeats for both men and women. Of the 202 total subjects in the study, 103 exhibited <22 CAG repeats (51 men and 52 women; 50.9%) and 99 exhibited ≥22 repeats (61 men and 38 women; 49.1%). Women heterozygous for one short and one long allele were not included in the analysis. No significant differences existed between the two repeat groups for any physical characteristic. Similar to the STORM cohort and contrary to our hypothesis, men with ≥22 repeats exhibited significantly greater FFM (57.2 ± 1.1 vs. 53.8 ± 1.1 kg; P = 0.047) and relative FFM (18.7 ± 0.3 vs. 17.8 ± 0.3 kg/m²; P = 0.050) than men with fewer repeats. There were no significant differences observed for women. No significant differences were observed in muscle strength between AR repeat groups for either men or women (Table 4).

Testosterone. No significant differences were observed for either total or bioavailable testosterone in the STORM cohort when AR CAG repeat length was analyzed by the median value of 22. However, when repeat length was analyzed as tertiles (short alleles, <21 repeats, n = 71; medium alleles, 21–23 repeats, n = 113; long alleles, ≥23 repeats, n = 110), the relationship between testosterone and CAG repeat number approached statistical significance, with the greatest repeat tertile (≥23) demonstrating the highest total testosterone (<21: 432.4 ± 16.8; 21–23: 414.3 ± 13.2; ≥23: 448.3 ± 13.4 ng/dl; P = 0.072) and bioavailable testosterone (124.1 ± 4.8, 56.1 ± 2.2 ng/dl; P = 0.002).
Table 4. Concentric and eccentric knee extensor peak torque values by AR CAG repeat number in BLSA men and women

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th></th>
<th></th>
<th></th>
<th>Women</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;22</td>
<td>≥22</td>
<td>P value</td>
<td></td>
<td>&lt;22</td>
<td>≥22</td>
<td>P value</td>
<td></td>
</tr>
<tr>
<td>Concentric (0.52 rad/s)</td>
<td>183.7±4.4</td>
<td>177.8±4.5</td>
<td>0.261</td>
<td>119.4±3.6</td>
<td>116.4±4.2</td>
<td>0.859</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentric (3.14 rad/s)</td>
<td>124.6±3.0</td>
<td>121.1±3.1</td>
<td>0.418</td>
<td>78.1±2.4</td>
<td>76.0±2.7</td>
<td>0.565</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eccentric (0.52 rad/s)</td>
<td>236.2±6.5</td>
<td>230.1±6.5</td>
<td>0.508</td>
<td>157.9±5.0</td>
<td>147.1±5.8</td>
<td>0.164</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eccentric (3.14 rad/s)</td>
<td>241.0±6.4</td>
<td>242.4±6.2</td>
<td>0.879</td>
<td>164.2±5.7</td>
<td>155.9±6.7</td>
<td>0.356</td>
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</table>

Values are means ± SE in Nm. Data are corrected for age, height, and physical activity. There were no statistically significant differences between repeat groups for both men and women.

Although the mechanism underlying the observed association requires further study, we observed higher testosterone levels in men with longer alleles, consistent with our findings of greater FFM among individuals with longer alleles. These findings, if verified, may have important implications for the prevention of sarcopenia and frailty in aging adults.

The association between AR CAG repeat number and FFM was in the opposite direction of our a priori hypothesis. Numerous reports have shown that a greater number of CAG repeats in the human AR gene causes a linear decrease in receptor transactivation function (6, 7, 20, 43). Thus we hypothesized that more CAG repeats would result in reduced activity of the androgen-AR pathway, with negative consequences for muscle mass. One possible explanation for our results is that a reduction in AR activity in the long allele length group modulates the level of negative feedback on the hypothalamic-pituitary-testicular axis, thus resulting in higher serum androgen levels. Higher testosterone levels have been associated with higher AR CAG repeat number in some, but not all, studies (22, 29, 47). For example, Krithivas et al. (22) reported in a longitudinal study of aging in older men that testosterone levels, while similar at baseline regardless of CAG repeat number, were significantly higher at follow up in men with higher CAG repeat number compared with men with fewer CAG repeats. These results are consistent with our cross-sectional results in the BLSA cohort, where a significant gene-by-age interaction was observed, such that older but not younger men in the ≥22 CAG repeat group exhibited higher testosterone levels compared with the men with <22 repeats. Krithivas et al. suggest that greater AR CAG repeat number decreases AR activity in the hypothalamus, resulting in decreased negative feedback, thereby increasing serum androgen levels. This effect on testosterone, albeit modest, would be genetically mediated and thus could have an impact on muscle over the lifespan. This suggestion is consistent with the findings of the present study, in which we observed higher levels of total testosterone as well as significantly greater total FFM and relative total FFM in men with greater repeat number compared with those with fewer repeats. Whether higher testosterone levels have a direct or indirect effect on muscle is uncertain and cannot be determined from the present study. The influence of testosterone on muscle mass may be indirect and mediated via an antiglucocorticoid effect (9, 10, 21), helping to diminish glucocorticoid-induced atrophy. For example, Danhaive and Rousseau (10) showed in rats that testosterone antagonizes the catabolic activity of endogenous glucocorticoids.

While we observed consistent associations between AR CAG repeat number and FFM in men from two independent
cohorts, we did not observe a similar association in women from the BLSA cohort. Our study was underpowered to specifically address sex differences in the association between the CAG repeat polymorphism and FFM, so this observation will require verification. Moreover, the study of the AR gene in women is complicated by the presence of two copies of the AR gene (i.e., two X chromosomes), one of which is randomly inactivated in each cell during development (25). Recent research has shown that both premenopausal and postmenopausal women with relatively few CAG repeats in the AR gene displayed higher levels of serum androgens than women with a greater number of CAG repeats (16, 49), findings opposite of what we observed in men in the present study. Whether the CAG repeat polymorphism has different actions and consequences in men compared with women is unclear, and additional work in this area is warranted.

The present results add the AR gene to a small but growing list of genes that have been tentatively identified as contributing to interindividual variation in skeletal muscle phenotypes, with anticipated future clinical significance for a variety of muscle-wasting conditions, especially sarcopenia. Not only will the importance of all of these genes need to be confirmed, but the interactions among them will also need to be examined. Moreover, for genes with a verified influence on muscle, potential interactions with therapeutic strategies (e.g., strength training or hormonal therapy) will need to be explored, as the ultimate goal of this research is to allow for optimization of individual prescriptions for maintaining muscle function throughout the age span.

In summary, we present data showing a significant association between AR CAG repeat number and FFM in men with corresponding differences in testosterone levels. Confirmation of the observed associations between AR CAG repeat number, testosterone levels, and FFM, as well as additional exploration of the mechanisms underlying these findings, is warranted.

GRANTS

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