Androgen receptor gene polymorphisms lean mass and performance in young men

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ABSTRACT

The exon-1 of the androgen receptor (AR) gene contains two repeat length polymorphisms which modify either the amount of AR protein inside the cell (GGN, polyglycine) or its transcriptional activity (CAGN, polyglutamine). Shorter CAG and/or GGN repeats were associated with stronger androgen signalling and vice versa. To test the hypothesis that CAG and GGN repeat AR polymorphisms affect muscle mass and various variables of muscular strength phenotype traits, the length of CAG and GGN repeats was determined by PCR and fragment analysis and confirmed by DNA sequencing of selected samples in 282 men (28.6±7.6 years). No significant differences in lean body mass or fitness were observed between the CAG short (CAGS) and long (CAGL) or GGN short (GGNS) and long (GGNL) groups, but a trend for a correlation was found for the GGN repeat and lean mass of the extremities (r=−0.11, p=0.06). In summary, the lengths of CAG and GGN repeat of the AR gene do not appear to influence lean mass or fitness in young men.

INTRODUCTION

Muscle mass and strength, as well as aerobic fitness (VO2max) are related to health and mortality.1 Muscle mass and strength is determined by environmental factors, principally endocrine, nutritional and mechanical loading, and by the genetic background.2 Gene polymorphisms, like those encoding for the insulin-like growth factor-1 (IGF-1),3 type I collagen (COLI A1),4 ciliary neurotrophic factor (CNTF),5 interleukin-6 (IL-6),6 the vitamin D receptor (VDR),7 IGF-2,8 resistin (RETN)9 and androgen receptor (AR).10 have an influence on either muscle mass or strength.

The AR gene is located to the X chromosome (q11.2–q12), and contains eight exons. The exon 1 contains a polyglutamine tract encoded by CAG repeats and a polyglycine tract (GGN) encoded by (GGT)6GGG(GGT)6(GGC)8. Polymorphic tracts are close to the region encoding the transactivation-1 domain of the AR protein.11 The CAG and GGN polymorphisms of the AR gene are related to incidence of prostatic cancer, breast cancer, plasma hormone levels and other metabolic, cardiovascular and even mental diseases.12–15 The polyglutamine repeat has an average length of 22 amino acids (range: 8–35). Short CAG repeats are associated with increased AR transactivation activity and stronger transcriptional potential.16 The CAG polymorphisms are associated with the fat-free mass phenotype in healthy elders.10 However, it remains to be established if the AR polymorphism influences muscle mass and fitness in young adults.

The polyglutamine repeat length of AR ranges from 10 to 30.17 Short GGN repeats are associated with increased AR protein content in cell cultures that may in turn enhance the response to androgen stimulation.18 It remains unknown if a short GGN repeat number is associated to increased muscle mass or strength in humans.

The aim of this study was to determine if AR polymorphisms are associated to muscle mass and physical fitness in adult men. We tested the hypothesis of whether men with short CAG and/or short GGN repeats have greater fat-free mass and muscle mass, and, therefore, greater strength and muscle power, than those harbouring long CAG and/or long GGN repeats. Since studies in cell culture and animal models have shown that androgen—AR signalling pathway increases the expression of slow-twitch-specific skeletal muscle proteins leading to a more oxidative phenotype,19 we also studied whether AR polymorphisms have an effect on aerobic power (VO2max) in humans. This information may be useful to elaborate genetic profiles like those recently proposed by Lucia et al.20–22 to explain individual variations in human physical performance.

METHODS

Subjects

Two-hundred and eighty-two Caucasian men participated in the study. They were recruited from physically active university students, sports clubs and local police officers in Gran Canaria (Spain). Recruitment started in February 2003 and extended to June 2007. The health status of each participant was established by a medical history and physical examination. Subjects taking any kind of medications or having any chronic disease or hypertension were excluded. The study was performed in accordance with the Helsinki Declaration of 1975 as regards the conduct of clinical research, being approved by the Ethical Committee of the University of Las Palmas de Gran Canaria. All volunteers provided their written informed consent before participation in the study.
Tests
Tests were carried out over 4 days. The first testing day started with a 20-ml blood sample which was obtained from an antecubital vein in the supine position, between 7:30 and 8:30. Body composition, jumping performance and maximal isometric force was tested on the second day. The last 2 days were used to assess sprint performance and anaerobic capacity, as well as maximal aerobic power (VO$_2$max).

Body composition
Whole body composition was assessed by dual-energy x-ray absorptiometry (DXA; QDR-1500; Hologic Corp., software version 7.10, Waltham, Massachusetts, USA) as reported in Perez-Gomez et al. Upper and lower limb lean mass (kg) was calculated from the regional analysis of the whole body scan, which gives a valid and reliable estimate of muscle mass in the extremities.26

Vertical jump performance and running sprint tests
The forces generated during vertical jumps were measured with a force platform (Kistler, Winterthur, Switzerland), as reported in Ara et al. Two kinds of jumps were performed: squat jump, in which countermovement was not permitted, and countermovement jump, from standing position subjects were asked to perform a countermovement, intending to reach knee bending angles of around 90° just before impulsion.

Aerobic maximal power
The maximal oxygen uptake (VO$_2$max) was estimated using the maximal multistage 20-m shuttle run. The time during which the subjects were able to run for was recorded to calculate VO$_2$max.

CAG and GGN repeat polymorphisms
DNA was extracted from blood samples (200 µl) using High Pure PCR Template Preparation Kits (Roche Applied Science). To determine the length of the CAG and GGN repeats, the corresponding regions located on the exon 1 of the AR gene (Genbank accession no. M27423) were amplified using two pairs of primers whose sequences have been previously reported. One primer from each pair was marked with fluorescent dye (FAM or VIC). Amplification was performed in a 25-µl reaction volume, containing 50 ng of genomic DNA, 200 µM of each deoxynucleotide triphosphate, 1× FastStart Taq DNA polymerase buffer (Roche Applied Science, Mannheim, Germany), 1× GC-rich solution buffer (Roche Applied Science) and 1 U of FastStart Taq DNA polymerase (Roche Applied Science). The concentration of each pair of primers was 1.2 and 1.5 µM for the amplification of the CAG and GGN repeats, respectively. PCR conditions were 30 cycles of 95°C for 1 min, 55°C for 2 min and 72°C for 2 min for GGN amplification. Each PCR was initiated with a denaturation step at 95°C for 5 min and terminated with an extension step at 72°C for 5 min. The PCR product was diluted 1:100 in distilled water, and 1 µl of the dilution was mixed with 10 µl of formamide and 0.3 µl of GeneScan 500 LIZ Size Standard (Applied Biosystems, Warrington, UK), denatured at 98°C for 5 min and cooled on ice. Fragment separation was performed by automated capillary electrophoresis, using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems), and the length was determined with GeneScan Analysis Software (version 3.7; Applied Biosystems). Internal standards supplied by the manufacturer were used for quality control. We blindly repeated the genotype analysis in 54 of the samples, and the results were completely coincident. The fragments size was confirmed by sequencing 48 DNA samples harbouring different size alleles for both repeats by using the BigDye Terminator Sequencing Kit (Applied Biosystem) at University of Las Palmas Sequencing Facility. Genotyping was performed specifically for research purposes based on the hypothesis that the aforementioned polymorphisms may influence VO$_2$max, lean mass and muscle strength. The genotype data of the subjects were not previously analysed for other non-research purposes and as such were not presented a posteriori for the present paper. The researchers in charge of genotyping were totally blinded to the subjects’ identities, that is, blood samples were tracked with a force platform (Kistler, Winterthur, Switzerland), as reported in Ara et al. Two kinds of jumps were performed: squat jump, in which countermovement was not permitted, and countermovement jump, from standing position subjects were asked to perform a countermovement, intending to reach knee bending angles of around 90° just before impulsion.

Table 1 Subject’s body composition, anthropometrics, physical activity and fitness (mean±SD)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Mean±SD</th>
<th>n</th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28.8±7.6</td>
<td>282</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>176.8±5.5</td>
<td>282</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>79.2±10.3</td>
<td>282</td>
</tr>
<tr>
<td>Percentage of body fat</td>
<td>19.3±7.3</td>
<td>282</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>59.5±5.6</td>
<td>282</td>
</tr>
<tr>
<td>Lean body mass/HT$^2$ (kg/m$^2$)</td>
<td>19.0±1.5</td>
<td>282</td>
</tr>
<tr>
<td>Lean mass arms (kg)</td>
<td>6.7±0.9</td>
<td>282</td>
</tr>
<tr>
<td>Lean mass legs (kg)</td>
<td>19.8±2.2</td>
<td>282</td>
</tr>
<tr>
<td>Lean mass extremities (kg)</td>
<td>26.4±2.9</td>
<td>282</td>
</tr>
<tr>
<td>Lean mass arms/HT$^2$ (kg/m$^2$)</td>
<td>2.1±0.3</td>
<td>282</td>
</tr>
<tr>
<td>Lean mass legs/HT$^2$ (kg/m$^2$)</td>
<td>6.3±0.6</td>
<td>282</td>
</tr>
<tr>
<td>Lean mass extremities/HT$^2$ (kg/m$^2$)</td>
<td>8.4±0.8</td>
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</tr>
<tr>
<td>Sports history (years)</td>
<td>8.0±6.0</td>
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<tr>
<td>Jumping tests</td>
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<tr>
<td>SJHJ (m)</td>
<td>0.292±0.054</td>
<td>251</td>
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<tr>
<td>SJWmax (w)</td>
<td>3409±536</td>
<td>192</td>
</tr>
<tr>
<td>SJWmax/MML (w/kg)</td>
<td>173±19</td>
<td>192</td>
</tr>
<tr>
<td>CMJH (m)</td>
<td>0.331±0.061</td>
<td>252</td>
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<tr>
<td>CMJWmax (w)</td>
<td>3586±556</td>
<td>194</td>
</tr>
<tr>
<td>CMJWmax/MML (w/kg)</td>
<td>180±28</td>
<td>192</td>
</tr>
<tr>
<td>Strength</td>
<td></td>
<td></td>
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<tr>
<td>MVC (kgf)</td>
<td>106±21</td>
<td>237</td>
</tr>
<tr>
<td>MVC/MML (kgf/kg)</td>
<td>5.4±1.0</td>
<td>237</td>
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<tr>
<td>Running test</td>
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<td></td>
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<tr>
<td>$T_{30m}$ (s)</td>
<td>4.53±0.29</td>
<td>272</td>
</tr>
<tr>
<td>$T_{300m}$ (s)</td>
<td>50.17±8.65</td>
<td>271</td>
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<tr>
<td>Aerobic power</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO$_2$max (ml/kg/ml)</td>
<td>47.3±7.5</td>
<td>267</td>
</tr>
</tbody>
</table>

CMJH, jumping height in countermovement jumps; CMJWmax, maximal power in countermovement jumps; CMJWmax/MML, maximal power in countermovement jumps per kg of muscle mass in the lower extremities; HT, height; MVC, maximal isometric force in the squatting position; SJHJ, jumping height in squat jumps; SJWmax, maximal power in squat jumps; SJWmax/MML, maximal power in squat jumps per kg of muscle mass in the lower extremities (MML); $T_{30m}$ and $T_{300m}$, running time in the 30 and 300 m running sprint, respectively.
RESULTS

Subject's body composition, anthropometrics, physical activity and fitness are reported in table 1. The observed allele frequencies for AR CAG and GGN repeat numbers in the studied subjects are presented in fig. 1. There were 17 different CAG alleles (ranging from 15 to 35 repeats) and 14 GGN alleles, ranging from 12 to 28 repeats.

CAG repeat polymorphism

Subject's body composition, anthropometrics, physical activity and fitness in the GGN<sub>S</sub> and GGN<sub>L</sub> groups are reported in table 2. The CAG polymorphism was not associated to any studied variable. No significant differences were found either in lean body mass or fitness between the CAG<sub>S</sub> and CAG<sub>L</sub> groups (table 2). There was no relationship between the length of the CAG repeat polymorphism and lean mass or physical fitness variables.

GGN repeat polymorphism

Subject's body composition, anthropometrics, physical activity and fitness in the GGN<sub>S</sub> and GGN<sub>L</sub> groups are reported in table 3. A trend for a significant inverse association between the logarithm of the length of the GGN polymorphism and the muscle mass of the extremities (MME) expressed as kg/height<sup>2</sup> was observed (MME=11.6–2.3×Lg GGN; R=0.11, p=0.06). The length of the GGN repeat polymorphism did not correlate with any of the physical fitness variables assessed.

Interaction between CAG and GGN repeat polymorphism

The body composition, anthropometrics, physical activity and fitness of men grouped as CAG<sub>S</sub>+GGN<sub>L</sub>, CAG<sub>S</sub>+GGN<sub>S</sub>, CAG<sub>L</sub>+GGN<sub>L</sub> and CAG<sub>L</sub>+GGN<sub>S</sub> are reported in table 4. Although men having the combination CAG<sub>S</sub> and GGN<sub>L</sub> jumped 9.0% higher than those having the combination CAG<sub>L</sub> and GGN<sub>L</sub> (table 4), this effect was not significant after accounting for multiple comparisons (p=0.13). Differences between allele combinations in other physical fitness and lean mass variables were not significant, even without accounting for multiple comparisons.

DISCUSSION

This study shows that in physically active young men, AR polyglycine and polyglutamine repeat polymorphisms have no influence on lean mass or fitness when studied alone. Although the subjects with the combination CAG<sub>S</sub> and GGN<sub>L</sub> jumped higher than those with the combination of CAG<sub>L</sub> and GGN<sub>L</sub>, this effect disappeared after accounting for multiple comparisons. However, we cannot rule out a potential type II error, implying that this effect needs to be verified in future studies.

In agreement with previous studies, we did not observe any association between height and length of the CAG repeat polymorphism in men.<sup>10</sup> Although the subjects having a CAG repeat number ≥22 had a 1.1% greater height<sup>2</sup>-adjusted lean body mass than the group with shorter alleles, this difference did not reach statistical significance. This is in contradiction to results from Walsh et al<sup>11</sup> that reported a 2% greater lean body mass in the subjects with a CAG repeat number ≥22, in a group of 294 men with a mean age of 73 years. The difference between both studies is likely due to the fact that our subjects were ascribed to the GGN<sub>S</sub> group if harbouring any of the following allele combinations: CAG<sub>S</sub>+GGN<sub>L</sub> (n=64), CAG<sub>L</sub>+GGN<sub>S</sub> (n=65), CAG<sub>S</sub>+GGN<sub>S</sub> (n=48) and CAG<sub>L</sub>+GGN<sub>S</sub> (n=87).

Mean values were compared using analysis of variance with two factors (CAG and GGN lengths), each with two levels (short and long repeat number). Pairwise comparisons were tested for statistical significance using the Bonferroni post hoc test. Lean mass was corrected for differences in height by dividing muscle mass by height.<sup>2 31</sup>
were much younger (29 years old) and had more appendicular muscle mass but lower height-2-adjusted whole body lean mass than the subjects studied by Walsh et al, suggesting that with ageing men may increase trunk lean mass, due to changes in other components of the trunk lean mass apart from the muscle tissue as demonstrated by using potassium whole body counting. \(^3^2\) No significant differences in appendicular muscle mass in men related to the hypothesis that this combination may have a favourable change muscle fibres in men. \(^3^8\) and 20 weeks treatment with testosterone enanthate did not increase androgen signalling may stimulate the expression of slow-twitch-specific skeletal muscle proteins while inhibiting fast-twitch-specific skeletal muscle proteins. \(^3^9\) However, there are no sex differences in muscle fibre types in humans, \(^3^7\) and 20 weeks treatment with testosterone enanthate did not change muscle fibres in men. \(^3^0\)

### Acknowledgements

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### Ethics approval

This study was conducted with the approval of the University of Las Palmas de Gran Canaria.
Table 4  Body composition, anthropometrics, physical activity and fitness in men harbouring the microsatellite combinations CAG\textsubscript{S}+GGN\textsubscript{L}, CAG\textsubscript{S}+GGN\textsubscript{L}, CAG\textsubscript{S}+GGN\textsubscript{L} and CAG\textsubscript{L}+GGN\textsubscript{S} (mean±SD)

<table>
<thead>
<tr>
<th></th>
<th>CAG\textsubscript{S}+GGN\textsubscript{L}</th>
<th>n</th>
<th>CAG\textsubscript{S}+GGN\textsubscript{L}</th>
<th>n</th>
<th>CAG\textsubscript{S}+GGN\textsubscript{L}</th>
<th>n</th>
<th>CAG\textsubscript{L}+GGN\textsubscript{S}</th>
<th>n</th>
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<tr>
<td>Age</td>
<td>29.9±8.1</td>
<td>48</td>
<td>28.1±6.9</td>
<td>87</td>
<td>28.5±8.5</td>
<td>64</td>
<td>29.2±7.3</td>
<td>83</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>176.8±6.0</td>
<td>48</td>
<td>176.0±5.2</td>
<td>87</td>
<td>177.3±5.4</td>
<td>64</td>
<td>177.3±5.8</td>
<td>83</td>
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<tr>
<td>Body mass (kg)</td>
<td>80.0±12.8</td>
<td>48</td>
<td>76.9±9.9</td>
<td>87</td>
<td>78.3±9.4</td>
<td>64</td>
<td>78.2±9.7</td>
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<tr>
<td>Percentage of body fat (%)</td>
<td>20.6±8.6</td>
<td>87</td>
<td>19.5±6.2</td>
<td>64</td>
<td>19.1±7.3</td>
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<td>18.5±7.6</td>
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<tr>
<td>Lean body mass (kg)</td>
<td>59.6±5.3</td>
<td>48</td>
<td>58.5±6.2</td>
<td>87</td>
<td>59.9±5.2</td>
<td>64</td>
<td>60.3±5.5</td>
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<tr>
<td>Lean mass arms (kg)</td>
<td>6.7±0.8</td>
<td>64</td>
<td>6.5±1.0</td>
<td>83</td>
<td>6.7±0.9</td>
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<td>6.8±0.9</td>
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<tr>
<td>Lean mass legs (kg)</td>
<td>19.6±2.0</td>
<td>87</td>
<td>19.5±2.4</td>
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<td>20.1±2.2</td>
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<td>19.9±2.1</td>
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<tr>
<td>Lean mass extremities (kg)</td>
<td>26.3±2.7</td>
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<td>25.9±3.3</td>
<td>87</td>
<td>26.6±2.9</td>
<td>64</td>
<td>26.7±2.7</td>
<td>83</td>
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<tr>
<td>Lean mass extremities (kg/m\textsuperscript{2})</td>
<td>2.1±0.3</td>
<td>48</td>
<td>2.1±0.4</td>
<td>87</td>
<td>2.1±0.3</td>
<td>64</td>
<td>2.2±0.3</td>
<td>83</td>
</tr>
</tbody>
</table>
| Maximal power in countermovement jumps per kg of muscle mass in the lower extremities; Ht, height; MVC, maximal isometric force in the squatting position; CMJJH, jumping height in squat jumps; SJMax, maximal power in squat jumps; SJWmax, maximal power in countermovement jumps per kg of muscle mass in the lower extremities

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Patient consent
Obtained.

REFERENCES

Take home message

The length of CAG and GGN repeat of the AR gene do not appear to influence lean mass or fitness in young men. Additional studies are required to test if men harbouring the combination CAG₃ and GGN₃ have more jumping capacity.

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