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Androgen receptor gene microsatellite polymorphism is associated with muscle mass and strength in bodybuilders and power athlete status

Running title: *AR* (CAG)_n polymorphism, muscle traits and athlete status

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ABSTRACT

Background: The androgen receptor (*AR*) gene contains a polymorphic trinucleotide (CAG) microsatellite repeat sequence (short or long alleles) that has been associated with fat-free mass in untrained men, which needs to be replicated in athletic cohorts. **Aim:** The purpose of this study was to explore the *AR* (CAG)_n polymorphism in trained individuals. **Subjects and methods:** A total of 61 professional bodybuilders (40 males, 21 females), 73 elite male sprinters and weightlifters and 186 male controls were enrolled in this study. The influence of the *AR* (CAG)_n polymorphism on muscle mass and strength was assessed in bodybuilders, while the frequencies of *AR* (CAG)_n alleles were compared between power athletes and non-athletes. **Results:** The polymorphism was associated with anthropometric and strength measurements in bodybuilders of both genders. Those with ≥ 21 CAG repeats (i.e., carriers of long alleles) exhibited greater ($P < 0.05$) body mass index, absolute muscle mass, arm/thigh circumference and upper/lower limb strength compared to those with < 21 CAG repeats. Furthermore, carriers of ≥ 21 CAG repeats were more frequent among power athletes compared to controls ($P=0.0076$). **Conclusions:** Long alleles of the *AR* (CAG)_n polymorphism were associated with greater muscle mass and strength in bodybuilders, and power athlete status.

Keywords: anthropometrics, genetics, hypertrophy, skeletal muscle, sprinters, weightlifters

Introduction

Androgens are steroid hormones synthesized from cholesterol that are biologically diverse targeting both reproductive and non-reproductive tissues, including anabolic actions on several body structures. After conversion of cholesterol to pregnenolone, several pathway permutations are possible, but all lead to the conversion of androstenedione, which is then converted to testosterone (Hooper et al. 2017). Testosterone or precursor androgens (e.g., dihydrotestosterone) exert their physiological effects largely by binding to the androgen receptor (AR), particularly in skeletal muscle (Dubois et al. 2012), where they have a well-known anabolic effect (Bhasin et al. 2001; Bhasin et al. 2005).

In the absence of ligands, the AR is confined to sarcoplasm in a multi-heteromeric inactive complex with accessory heat shock proteins (Georget et al. 2002). The steroidal nature of androgens allows them to cross the lipid bilayer of the sarcolemma to form a complex with the ARs and ultimately enhance gene transcription (Beato and Klug 2000). The molecular interaction with androgens induces the dissociation of the AR from the accessory proteins, its dimerization and translocation to the nucleus, where it binds to androgen response elements (AREs) at specific DNA sites, stimulating the transcription of target genes (Palazzolo et al. 2008). This mechanism represents the classical genomic pathway for the action of androgens in skeletal muscle, but the androgen–AR complex can also interfere with other signaling pathways via non-genomic actions. While the genomic pathway encodes muscle-specific transcription factors, enzymes and structural proteins, non-genomic actions are faster and can translate signals on muscle through activating specific kinases (Dubois et al. 2012) and triggering an increase in intracellular calcium (Ca^{2+}) levels (Estrada et al. 2003). Collectively, these mechanisms have the potential to influence muscle biology.

The AR protein is structurally composed of three functional domains: the COOH-terminal domain, which contains the ligand-binding domain; the central region, which

contains the DNA-binding domain, and the NH₂-terminal domain, which becomes active after ligand-binding interaction and through protein-protein interactions is responsible for the transcriptional activation of androgen-responsive genes (Palazzolo et al. 2008). The AR gene is localized in the long arm of the X chromosome, and contains a polymorphic trinucleotide CAG (Cytosine, Adenine, Guanine) microsatellite repeat sequence in exon 1, which normally ranges from 8 to 37 CAG repeats (Ackerman et al. 2012). This microsatellite repeat sequence encodes for a polyglutamine (PolyGln) chain in the AR NH₂-terminal domain, which has a variable length based on the number of CAG repeats (Figure 1).

[Figure 1 near here]

Androgen binding to the AR induces a conformational change in the structure of the receptor that facilitates molecular communication between the NH₂-terminal domain and the COOH-terminal domain, generating a (N/C) terminus interaction that is required for AR dimerization and fundamental for the binding to AREs (He et al. 1999). DNA binding causes the N/C interaction to be lost, allowing the recruitment of coactivators to initiate transcription (van Royen et al. 2012). A longer PolyGln chain may influence N/C interaction and the ability to recruit coregulators and components of the AR-mediated transcription machinery (Buchanan et al. 2004). *In vitro* analyzes have shown that the elongation of the PolyGln chain results in a linear decrease in the AR transcriptional competence in mammalian cell lines (Chamberlain et al. 1994; Kazemi-Esfarjani et al. 1995; Tut et al. 1997; Irvine et al. 2000). However, this effect has been shown to be cell (or tissue) specific (Beilin et al. 2000), presumably due to distinct profiles of AR coregulator proteins (Heemers and Tindall 2007). For example, while the levels of AR-mediated mRNA in prostate cells appear to correlate

inversely with PolyGln length (Albertelli et al. 2006), the opposite was seen in myoblasts (Sheppard et al. 2011).

Sheppard et al. (2011) identified a positive relationship between the number of CAG repeats and transcriptional activity in testosterone treated C2C12 cells. Moreover, the shorter CAG repeat length (with lower AR transcriptional activity) showed reduced differentiation capacity (Sheppard et al. 2011), which would likely result in altered muscle morphology and lower strength production in vivo (Chambon et al. 2010). However, studies exploring the association between the *AR* (CAG)_n polymorphism and muscle variables in humans have shown conflicting results (Walsh et al. 2005; Campbell et al. 2009; Nielsen et al. 2010) or found no association (Guadalupe-Grau et al. 2011; Folland et al. 2012; De Naeyer et al. 2014). Walsh et al. (2005) were the first to show that 294 healthy Caucasian men (aged 50-93 years) with ≥ 22 CAG repeats exhibited significantly greater fat-free mass than men with < 22 CAG repeats, which was replicated in an independent cohort composed of 112 Caucasian men (mean age 57.4 ± 1.6 years). Campbell et al. (2009) also found that CAG repeat length was positively associated with fat-free mass in 156 Ariaal men of northern Kenya (aged 20-70 years). Conversely, Nielsen et al. (2010) reported that CAG repeat length correlated inversely with muscle area in 393 Danish men aged 20–29 years. The age difference between conflicting results suggests a gene-by-age interaction; however, none of the aforementioned studies evaluated well-trained individuals, which constantly stimulate increases in androgen levels (D'Andrea et al. 2020).

Therefore, the aim of the present study was to explore the influence of the *AR* (CAG)_n polymorphism on muscle mass and strength in a cohort of resistance-trained individuals (bodybuilders). In addition, allelic frequencies of the *AR* (CAG)_n polymorphism were compared between elite power athletes and non-athletes. We tested the hypothesis that longer alleles (≥ 21 CAG repeats) are associated with greater muscle mass and strength in

bodybuilders, as well as more frequent in power athletes. If the polymorphism is advantageous for the development of skeletal muscle, it will probably be more frequent among athletes.

Subjects and methods

Participants

A total of 320 Russian subjects (299 males, 21 females) participated in this study. Participants were divided into 3 subgroups: 61 professional bodybuilders (40 males, age 30.5 ± 8.6 years; 21 females, age 30.0 ± 8.0 years), 73 elite male (international-level) power athletes (35 sprinters (100-400 m runners) and 38 weightlifters; age 26.1 ± 4.3 years) and 186 male controls (age 23.7 ± 4.9 years). The characteristics of bodybuilders are provided in Table 1. All power athletes were participants in international competitions as national team members. None of athletes had ever tested positive for doping by a World Anti-Doping Agency (WADA) accredited laboratory. Controls (males, $n = 186$) were healthy unrelated Russians without any competitive sport experience (explored by survey). The bodybuilders, athletes and controls were all Caucasians.

[Table 1 near here]

Procedures

First, the influence of the AR (CAG)_n polymorphism on anthropometric and strength measurements of male and female bodybuilders were evaluated and compared between AR (CAG)_n alleles. Study participants were grouped and compared as harboring ≥ 21 (i.e., long alleles) or < 21 (i.e., short alleles) CAG repeat lengths. This cut-off value was chosen to make

equal sized sub-groups (i.e., 50.8% of bodybuilders were carriers of long alleles; 20 males, 11 females).

Second, a case-control association study was conducted to assess whether the frequency of the *AR* (CAG)_n polymorphism was more frequent in elite power athletes (i.e. sprinters, weightlifters and subgroup of bodybuilders) compared to non-athletic individuals (controls). The classification of sprinters and weightlifters as 'elite' was carried out based on the maximum competitive level reached in official international competitions (they were also at least prize winners in the national competitions). Only those athletes with superior physical fitness in their sports disciplines are expected to qualify for international-level competitions. Given that bodybuilding is not currently an Olympic sport, the elite level in bodybuilding was established as those with muscle mass and strength superior to their counterparts (i.e., those in the tertile with the highest strength and muscle mass values). Thus, the case-control study included only elite male athletes as cases (named as power athletes).

All procedures adopted in the study were approved by the Ethics Committee of the St Petersburg Research Institute of Physical Culture. The study complied with the guidelines set out in the Declaration of Helsinki and ethical standards in sport and exercise science research. Written informed consent was obtained from each participant.

Anthropometric measurements

Subjects were measured barefoot, wearing only underwear. Body mass was measured using a battery-operated digital scale (precision 50 g). Height was measured using an anthropometer (precision 0.5 cm). Chest, waist, arm, thigh and calf circumferences were measured using a plastic measuring tape (1 mm precision). Subcutaneous skinfolds (subscapular, biceps, triceps, abdomen, back, thigh and calf) were measured using a skinfold caliper (1 mm

precision). The estimation of muscle mass was performed using the formula proposed by Matiegka (1921).

Training parameters

Training parameters were self-reported (i.e., assessed by questionnaire). Training age was expressed as years of resistance training. Personal records in squat and bench press were recorded in kilograms.

Genotyping

Molecular genetic analysis was performed with DNA samples obtained from epithelial mouth cells by alkaline extraction or using a DNK-sorb-A sorbent kit according to the manufacturer's instruction (Central Research Institute of Epidemiology, Moscow, Russia), depending on the method of sample collection (buccal swab or scrape). Genotyping for the *AR* gene (CAG)_n polymorphism was performed by polymerase chain reaction (PCR) on a Tercyk thermal cycler (DNA Technology, Moscow, Russia). The DNA was amplified using two primers (5'-TCCAGAATCTGTTCCAGAGCGTGC-3' and 5'-GCTGTGAAGGTTGCTGTTCCCTCAT-3') (Litech, Moscow, Russia). The length of amplified fragments (\approx 260–280 base pairs) varied only by the number of CAG repeats. For accurate assessment of fragment length, the DNA fragments were run on a polyacrylamide gel (6%). The lengths of unknown PCR products were calculated using DNA λ -markers (Sibenzyme, Novosibirsk, Russia). All genotyping analyses were conducted in duplicates blind to subject identity.

Statistical Analyses

The relationship between *AR* (CAG)_n alleles and different phenotypes were performed using multiple regression analysis. Associations between *AR* (CAG)_n alleles and anthropometric measurements were adjusted for age and training experience, while associations between *AR* (CAG)_n alleles and strength measurements were adjusted for age, training experience and weight. Allelic frequencies between athletes and controls were compared using Chi² test. Statistical analyses were conducted using GraphPad InStat (GraphPad Software Inc., San Diego, CA). All data are presented as mean (SD). The significance level was set at $P < 0.05$.

Results

Anthropometric and strength measurements

The anthropometric and strength measurements of the bodybuilders with respect to their *AR* (CAG)_n polymorphism are shown in Table 2. Male bodybuilders with long alleles (50.0%) exhibited greater absolute muscle mass ($P = 0.046$) and upper limb strength (i.e., bench press; $P = 0.018$) compared to male bodybuilders with short alleles. The upper limb strength remained significant even after adjustment for covariates ($P = 0.038$). Moreover, after adjustment for covariates, male bodybuilders with long alleles exhibited greater BMI ($P = 0.048$) and mid-upper arm circumference ($P = 0.036$). Among male bodybuilders there was also a tendency for greater thigh circumference ($0.05 < P < 0.075$). Female bodybuilders with long alleles (52.4%) exhibited greater weight ($P = 0.027$), BMI ($P = 0.02$), thigh circumference ($P = 0.043$), upper limb strength ($P = 0.044$) and lower limb strength (i.e., squat; $P = 0.004$) compared to female bodybuilders with short alleles. Weight ($P = 0.034$), BMI ($P = 0.023$) and maximum strength of the lower limbs ($P = 0.021$) remained significant following adjustment for covariates.

[Table 2 near here]

The combined data of male and female bodybuilders (adjusted for covariates) showed that bodybuilders with long alleles exhibited greater BMI ($P = 0.007$), absolute muscle mass ($P = 0.019$), mid-upper arm circumference ($P = 0.009$), thigh circumference ($P = 0.012$), upper limb strength ($P = 0.008$) and lower limb strength ($P = 0.012$) compared to bodybuilders with short alleles.

Case-control association study

The number of *AR* CAG repeats ranged from 15 to 25. The frequency of the *AR* (CAG)_n alleles in male athletes and controls is shown in Table 3. It was identified that long alleles were over-represented in elite athletes, especially in weightlifters (odds ratio (OR) = 2.9; $P = 0.015$). Although there was a prevalence of long alleles in sprinters and elite bodybuilders compared to controls, this comparison did not reach the significance threshold. However, the pooled data of all male athletes (i.e., sprinters, weightlifters and elite bodybuilders) confirmed the higher prevalence of long alleles in athletes. The presence of long alleles was observed in 77.4 % of power athletes compared to only 60.8% of controls (OR = 2.2; $P = 0.0076$).

[Table 3 near here]

Discussion

This is the first study to demonstrate that the *AR* gene (CAG)_n polymorphism is associated with greater muscle mass and strength in bodybuilders, and power athlete status. In accord with the study's hypothesis, bodybuilders carriers of long alleles for the *AR* (CAG)_n polymorphism (i.e., ≥ 21 CAG repeats) have a higher BMI, particularly due to a greater muscle mass. The greater muscle mass among bodybuilders carriers of ≥ 21 (CAG)_n was

accompanied by a greater maximum strength in both upper and lower limbs. These findings suggest that the elongation of the PolyGln chain in the NH₂-terminal domain of the AR, in part, facilitates gains in muscle mass and strength in resistance trained-individuals, which can be an advantage for strength/power athletes. In fact, carriers of ≥ 21 (CAG)_n were more frequent in a group of male athletes composed of sprinters, weightlifters and elite bodybuilders compared to male controls.

A recent study showed that muscle AR content is an important variable in resistance training-induced hypertrophy in resistance-trained healthy men (Morton et al. 2018), but not in untrained subjects (Mobley et al. 2018). In an assessment of high- and low-responders to resistance exercise training, it was found that AR content was greater in highest-responders, which was correlated with changes in muscle mass (Morton et al. 2018). Indeed, many of AR target genes are involved in skeletal muscle growth and development (Wyce et al. 2010), and therefore, regulate muscle biology (Sculthorpe et al. 2012). AR-mediated signaling may play an indispensable role in training-related muscle hypertrophy (Basualto-Alarcon et al. 2013; Yin et al. 2020). Thus, it is expected that the AR with greater transcriptional competence will contribute more sharply to the muscular response to physical exercise, in particular resistance exercise.

In previous *in vitro* studies, the elongation of the PolyGln chain in the NH₂-terminal domain correlates with an increase in the AR transcriptional competence in skeletal muscle cells (Sheppard et al. 2011), but not in non-muscle cells (Chamberlain et al. 1994; Kazemi-Esfarjani et al. 1995; Tut et al. 1997). It is worth mentioning that the influence of AR (CAG)_n might be different between tissues. Although additional work is needed to support the *in vivo* effect of the AR (CAG)_n polymorphism on muscle cells, our findings of greater muscle mass (and strength) in bodybuilders with ≥ 21 (CAG)_n are in line with this theory. Similar to that previously observed in older men from two different populations (Walsh et al. 2005;

Campbell et al. 2009). It should be noted that in cohorts composed of older individuals, participants may be hypogonadal and, to some extent, sarcopenic, which can influence the association and make it difficult to compare these studies with ours. Anyway, our findings were not seen only in adult men, but in women as well—all well-trained athletes.

In addition to the association with fat-free mass, Walsh et al. (2005) also showed that men with ≥ 22 CAG repeats exhibited significantly greater testosterone levels than men with < 22 CAG repeats. It has been proposed that the *AR* (CAG)_n polymorphism modulates the hypothalamic-pituitary-gonadal axis (Simanainen et al. 2011). The elongation of the PolyGln chain appears to decrease negative feedback control of luteinizing hormone (LH) release, thereby increasing LH levels and stimulating higher testosterone levels (Crabbe et al. 2007). Thus, the weaker transcriptional activity in some cell lines seems to be compensated for by higher testosterone levels (Huhtaniemi et al. 2009). This can be seen in several, but not all, studies (Ryan et al. 2017b); however, it should be noted that all the most powered studies—with larger sample sizes (i.e., > 1800 subjects)—report a small but consistently positive effect of the *AR* (CAG)_n polymorphism on testosterone levels in men (Huhtaniemi et al. 2009; Lindstrom et al. 2010; Haring et al. 2012). A power analysis using the estimated effect of the polymorphism showed that a sample size of roughly 1,000 subjects would have an 80% chance of detecting an association between the length of the PolyGln chain and hormone levels in men (Ryan et al. 2017b), and for this reason in studies with a larger sample the effect is always observed. Furthermore, in a study of Croatian women with Polycystic Ovary Syndrome (PCOS), those with > 22 CAG repeats also exhibited significantly greater testosterone levels than those with ≤ 22 CAG repeats (Skrgetic et al. 2012), which is in agreement with some other studies in women (Zhang et al. 2013). We did not assess our subjects' hormone levels, but the proposal is that this small but consistent effect of the *AR* (CAG)_n on androgen levels may partly contribute to the development of androgen-associated

somatic traits, such as muscle mass and strength. Although the hypertrophic effects of exercise-induced endogenous hormonal elevations are not a consensus (Fink et al. 2018), testosterone levels were positively related to muscle cross-sectional area in physically active men (De Naeyer et al. 2014), and testosterone response to resistance exercise was shown to be related to muscle hypertrophy in resistance-trained men (Mangine et al. 2017). Moreover, higher testosterone levels may confer an athletic advantage in certain power disciplines for both men and women, especially in sprint sports (Bermon and Garnier 2017; Ahmetov et al. 2020).

We are aware of some studies that found no significant differences in muscle mass or strength between *AR* (CAG)_n alleles in adult men (Guadalupe-Grau et al. 2011; Folland et al. 2012; De Naeyer et al. 2014); however, it should be noted that only sedentary or physically active individuals with no history of strength or power training were assessed. The present study was the only one to evaluate well-trained individuals. It was shown in a previous study that in young adult men (20–22 years old) with higher testosterone levels, the elongation of the PolyGln chain was able to predict increases in muscle mass or muscle area, that is, when testosterone levels are high, the PolyGln length is positively associated with muscle traits (Ryan et al. 2017a). In this regard, regular physical training can be a powerful stimulus for the acute increase in total and free testosterone levels (D'Andrea et al. 2020). Thus, we speculate that the effect of the polymorphism may have been more evident in our subjects, as they are trained individuals.

Typical bodybuilding-type training protocols, which include large muscle groups with moderate intensity, high volume, and relatively short rest periods, as well as other moderate to high intensity exercise protocols (commonly used by power athletes) are generally effective in inducing acute testosterone responses (D'Andrea et al. 2020). Interestingly, following a bodybuilding-type training protocol (regarding training intensity, volume and density)

elevated testosterone concentrations enhances muscle AR content compared to a training protocol that does not raise testosterone concentrations (Spiering et al. 2009). Furthermore, individual changes in muscle AR content following a 16–21 week resistance training program correlated with changes in fat-free mass and muscle cross-sectional area (Ahtiainen et al. 2011; Mitchell et al. 2013). This will create a favorable molecular environment for muscle hypertrophy. Even in the absence of changes in muscle AR content, high-load resistance exercises can significantly increase the AR-DNA binding post-exercise, presumably through AR coactivating protein β -catenin (Cardaci et al. 2020). It is noteworthy that the AR (CAG)_n polymorphism did not interfere with the DNA binding capacity (Belikov et al. 2015) and the elongated PolyGln chain could regulate AR function by serving as flexible spacers to the biological activity of coregulator proteins (Buchanan et al. 2004), particularly in skeletal muscle where the PolyGln length can positively affect AR-mediated transcription (Sheppard et al. 2011). Therefore, it appears reasonable to surmise that AR-mediated transcription can be optimized after resistance training, particularly in carriers of longer AR (CAG)_n alleles.

Alternatively, carriers of longer AR (CAG)_n alleles can take advantage of mechanisms other than the binding of ARs directly to AREs, including stimulation of other hormonal signals (such as growth hormone and insulin-like growth factor 1) and intracellular signaling via non-genomic actions (Hooper et al. 2017). There is a cross-talk between these mechanisms and AR-mediated transcription (Norman et al. 2004), which can lead to muscle hypertrophy (Semsarian et al. 1999). In addition, non-genomic androgenic mechanisms can counteract fatigue and enhance muscle function through increases in inositol trisphosphate (IP₃) and Ca²⁺ released from the sarcoplasmic reticulum (Dent et al. 2012). This effect can be seen mainly during repeated high-intensity muscle contractions (Kabbara and Allen 1999) and thus be beneficial for power athletes, such as weightlifters and sprinters. Although these mechanisms have not been investigated, in the study by Ponce González et al. (2017) the

higher lean mass in the lower limbs of Caucasian healthy men with ≥ 24 CAG repeats (mean age 29.2 ± 7.1 years) explains, in part, their faster 300-m running sprint performance compared to those with ≤ 19 CAG repeats (mean age 33.2 ± 8.9 years). To the best of our knowledge, this was the only study that evaluated sprint performance in adults with different *AR* (CAG)_n alleles.

In line with the hypothesis that longer *AR* (CAG)_n alleles favours power performance, in the present study there was a significantly higher frequency of men with ≥ 21 CAG repeats in the power athlete group compared to the non-athlete (control) group. Individuals with ≥ 21 CAG repeats are 2.2 times more likely to be a power athlete. These findings certainly need to be replicated in independent and larger cohorts. The sample size of our case-control study prevents us from making definitive statements, but the data suggests that longer *AR* (CAG)_n alleles are preferred for strength and power athletes. Further molecular studies will be interesting to better explore the biological bases that support the differences between the *AR* (CAG)_n alleles, especially among trained subjects. We strongly suspect that many additional common polymorphisms, and probably rare mutations as well, will be shown to be associated with strength performance in due course (Moreland et al. 2020).

The present study has some limitations and therefore the findings should be interpreted with caution. The first issue is that there was no standardization of the training method of the subjects (particularly bodybuilders) from the stand point of load, volume, recovery and nutritional parameters. For instance, someone with a so-called favourable CAG repeat length who undertrains may have acquired less muscle mass than someone with a so-called unfavorable CAG repeat length simply due to a greater training approach. The other critical issue is that serum total and free testosterone was not assessed. In the event that some of bodybuilders had been or were taking anabolic steroids, this may also have masked the genetic propensity. The replication of our findings is of paramount importance. Also, it should

be noted that other polymorphisms in the *AR* gene and possible post-translational modifications may potentially affect training outcomes; however, the present study provides an initial support for the *AR* (CAG)_n polymorphism as a contributing marker to the interindividual variability of skeletal muscle traits in trained individuals.

In conclusion, longer alleles for the *AR* (CAG)_n polymorphism were associated with greater muscle mass and strength in bodybuilders and power athlete status. An increase, but still within the normal physiological range, of CAG repeats in the NH₂-terminal domain of the *AR* gene can contribute to the improvement of muscle traits in resistance-trained individuals, and increase the predisposition for strength and power sports. Not only the importance of this polymorphism needs to be confirmed in larger samples, but its interaction with other key polymorphisms needs to be assessed.

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Disclosure statement

The authors report no conflicts of interest.

References

- Ackerman CM, Lowe LP, Lee H, Hayes MG, Dyer AR, Metzger BE, Lowe WL, Urbanek M. 2012. Ethnic variation in allele distribution of the androgen receptor (*AR*) (CAG)_n repeat. *J Androl.* 33(2):210-215.
- Ahmetov, II, Stepanova AA, Biktagirova EM, Semenova EA, Shchuplova IS, Bets LV, Andryushchenko LB, Borisov OV, Andryushchenko ON, Generozov EV et al. 2020. Is testosterone responsible for athletic success in female athletes? *J Sports Med Phys Fitness.* 60(10):1377-1382.

- Ahtiainen JP, Hulmi JJ, Kraemer WJ, Lehti M, Nyman K, Selanne H, Alen M, Pakarinen A, Komulainen J, Kovanen V et al. 2011. Heavy resistance exercise training and skeletal muscle androgen receptor expression in younger and older men. *Steroids*. 76(1-2):183-192.
- Albertelli MA, Scheller A, Brogley M, Robins DM. 2006. Replacing the mouse androgen receptor with human alleles demonstrates glutamine tract length-dependent effects on physiology and tumorigenesis in mice. *Mol Endocrinol*. 20(6):1248-1260.
- Basualto-Alarcon C, Jorquera G, Altamirano F, Jaimovich E, Estrada M. 2013. Testosterone signals through mTOR and androgen receptor to induce muscle hypertrophy. *Med Sci Sports Exerc*. 45(9):1712-1720.
- Beato M, Klug J. 2000. Steroid hormone receptors: an update. *Hum Reprod Update*. 6(3):225-236.
- Beilin J, Ball EM, Favaloro JM, Zajac JD. 2000. Effect of the androgen receptor CAG repeat polymorphism on transcriptional activity: specificity in prostate and non-prostate cell lines. *J Mol Endocrinol*. 25(1):85-96.
- Belikov S, Bott LC, Fischbeck KH, Wrangé O. 2015. The polyglutamine-expanded androgen receptor has increased DNA binding and reduced transcriptional activity. *Biochem Biophys Rep*. 3:134-139.
- Bermon S, Garnier PY. 2017. Serum androgen levels and their relation to performance in track and field: mass spectrometry results from 2127 observations in male and female elite athletes. *Br J Sports Med*. 51(17):1309-1314.
- Bhasin S, Woodhouse L, Casaburi R, Singh AB, Bhasin D, Berman N, Chen X, Yarasheski KE, Magliano L, Dzekov C et al. 2001. Testosterone dose-response relationships in healthy young men. *Am J Physiol Endocrinol Metab*. 281(6):E1172-1181.
- Bhasin S, Woodhouse L, Casaburi R, Singh AB, Mac RP, Lee M, Yarasheski KE, Sinha-Hikim I, Dzekov C, Dzekov J et al. 2005. Older men are as responsive as young men to the anabolic effects of graded doses of testosterone on the skeletal muscle. *J Clin Endocrinol Metab*. 90(2):678-688.
- Buchanan G, Yang M, Cheong A, Harris JM, Irvine RA, Lambert PF, Moore NL, Raynor M, Neufing PJ, Coetzee GA et al. 2004. Structural and functional consequences of glutamine tract variation in the androgen receptor. *Hum Mol Genet*. 13(16):1677-1692.
- Campbell BC, Gray PB, Eisenberg DT, Ellison P, Sorenson MD. 2009. Androgen receptor CAG repeats and body composition among African men. *Int J Androl*. 32(2):140-148.
- Cardaci TD, Machek SB, Wilburn DT, Heileson JL, Willoughby DS. 2020. High-Load Resistance Exercise Augments Androgen Receptor–DNA Binding and Wnt/B-Catenin

- Signaling without Increases in Serum/Muscle Androgens or Androgen Receptor Content. *Nutrients*. 12:3829.
- Chamberlain NL, Driver ED, Miesfeld RL. 1994. The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. *Nucleic Acids Res*. 22(15):3181-3186.
- Chambon C, Duteil D, Vignaud A, Ferry A, Messaddeq N, Malivindi R, Kato S, Chambon P, Metzger D. 2010. Myocytic androgen receptor controls the strength but not the mass of limb muscles. *Proc Natl Acad Sci U S A*. 107(32):14327-14332.
- Crabbe P, Bogaert V, De Bacquer D, Goemaere S, Zmierzak H, Kaufman JM. 2007. Part of the interindividual variation in serum testosterone levels in healthy men reflects differences in androgen sensitivity and feedback set point: contribution of the androgen receptor polyglutamine tract polymorphism. *J Clin Endocrinol Metab*. 92(9):3604-3610.
- D'Andrea S, Spaggiari G, Barbonetti A, Santi D. 2020. Endogenous transient doping: physical exercise acutely increases testosterone levels-results from a meta-analysis. *J Endocrinol Invest*. 43(10):1349-1371.
- De Naeyer H, Bogaert V, De Spaey A, Roef G, Vandewalle S, Derave W, Taes Y, Kaufman JM. 2014. Genetic variations in the androgen receptor are associated with steroid concentrations and anthropometrics but not with muscle mass in healthy young men. *PLoS One*. 9(1):e86235.
- Dent JR, Fletcher DK, McGuigan MR. 2012. Evidence for a Non-Genomic Action of Testosterone in Skeletal Muscle Which may Improve Athletic Performance: Implications for the Female Athlete. *J Sports Sci Med*. 11(3):363-370.
- Dubois V, Laurent M, Boonen S, Vanderschueren D, Claessens F. 2012. Androgens and skeletal muscle: cellular and molecular action mechanisms underlying the anabolic actions. *Cell Mol Life Sci*. 69(10):1651-1667.
- Estrada M, Espinosa A, Muller M, Jaimovich E. 2003. Testosterone stimulates intracellular calcium release and mitogen-activated protein kinases via a G protein-coupled receptor in skeletal muscle cells. *Endocrinology*. 144(8):3586-3597.
- Fink J, Schoenfeld BJ, Nakazato K. 2018. The role of hormones in muscle hypertrophy. *Phys Sportsmed*. 46(1):129-134.
- Folland JP, Mc Cauley TM, Phypers C, Hanson B, Mastana SS. 2012. The relationship of testosterone and AR CAG repeat genotype with knee extensor muscle function of young and older men. *Exp Gerontol*. 47(6):437-443.

- Georget V, Terouanne B, Nicolas JC, Sultan C. 2002. Mechanism of antiandrogen action: key role of hsp90 in conformational change and transcriptional activity of the androgen receptor. *Biochemistry*. 41(39):11824-11831.
- Guadalupe-Grau A, Rodriguez-Gonzalez FG, Dorado C, Olmedillas H, Fuentes T, Perez-Gomez J, Delgado-Guerra S, Vicente-Rodriguez G, Ara I, Guerra B et al. 2011. Androgen receptor gene polymorphisms lean mass and performance in young men. *Br J Sports Med*. 45(2):95-100.
- Haring R, Ernst F, Schurmann C, Homuth G, Volker U, Volzke H, Nauck M, Wallaschofski H. 2012. The androgen receptor CAG repeat polymorphism as a risk factor of low serum testosterone and its cardiometabolic effects in men. *Int J Androl*. 35(4):511-520.
- He B, Kempainen JA, Voegel JJ, Gronemeyer H, Wilson EM. 1999. Activation function 2 in the human androgen receptor ligand binding domain mediates interdomain communication with the NH(2)-terminal domain. *J Biol Chem*. 274(52):37219-37225.
- Heemers HV, Tindall DJ. 2007. Androgen receptor (AR) coregulators: a diversity of functions converging on and regulating the AR transcriptional complex. *Endocr Rev*. 28(7):778-808.
- Hooper DR, Kraemer WJ, Focht BC, Volek JS, DuPont WH, Caldwell LK, Maresh CM. 2017. Endocrinological Roles for Testosterone in Resistance Exercise Responses and Adaptations. *Sports Med*. 47(9):1709-1720.
- Huhtaniemi IT, Pye SR, Limer KL, Thomson W, O'Neill TW, Platt H, Payne D, John SL, Jiang M, Boonen S et al. 2009. Increased estrogen rather than decreased androgen action is associated with longer androgen receptor CAG repeats. *J Clin Endocrinol Metab*. 94(1):277-284.
- Irvine RA, Ma H, Yu MC, Ross RK, Stallcup MR, Coetzee GA. 2000. Inhibition of p160-mediated coactivation with increasing androgen receptor polyglutamine length. *Hum Mol Genet*. 9(2):267-274.
- Kabbara AA, Allen DG. 1999. The role of calcium stores in fatigue of isolated single muscle fibres from the cane toad. *J Physiol*. 519 Pt 1:169-176.
- Kazemi-Esfarjani P, Trifiro MA, Pinsky L. 1995. Evidence for a repressive function of the long polyglutamine tract in the human androgen receptor: possible pathogenetic relevance for the (CAG)_n-expanded neuropathies. *Hum Mol Genet*. 4(4):523-527.
- Lindstrom S, Ma J, Altshuler D, Giovannucci E, Riboli E, Albanes D, Allen NE, Berndt SI, Boeing H, Bueno-de-Mesquita HB et al. 2010. A large study of androgen receptor germline variants and their relation to sex hormone levels and prostate cancer risk. *Results*

- from the National Cancer Institute Breast and Prostate Cancer Cohort Consortium. *J Clin Endocrinol Metab.* 95(9):E121-127.
- Mangine GT, Hoffman JR, Gonzalez AM, Townsend JR, Wells AJ, Jajtner AR, Beyer KS, Boone CH, Wang R, Miramonti AA et al. 2017. Exercise-Induced Hormone Elevations Are Related to Muscle Growth. *J Strength Cond Res.* 31(1):45-53.
- Matiegka J. 1921. The testing of physical efficiency. *Am J Phys Anthropol.* 4(3):223–230.
- Mitchell CJ, Churchward-Venne TA, Bellamy L, Parise G, Baker SK, Phillips SM. 2013. Muscular and systemic correlates of resistance training-induced muscle hypertrophy. *PLoS One.* 8(10):e78636.
- Mobley CB, Haun CT, Roberson PA, Mumford PW, Kephart WC, Romero MA, Osburn SC, Vann CG, Young KC, Beck DT et al. 2018. Biomarkers associated with low, moderate, and high vastus lateralis muscle hypertrophy following 12 weeks of resistance training. *PLoS One.* 13(4):e0195203.
- Moreland E, Borisov OV, Semenova EA, Larin AK, Andryushchenko ON, Andryushchenko LB, Generozov EV, Williams AG, Ahmetov II. 2020. Polygenic Profile of Elite Strength Athletes. *J Strength Cond Res.* DOI: 10.1519/JSC.0000000000003901.
- Morton RW, Sato K, Gallagher MPB, Oikawa SY, McNicholas PD, Fujita S, Phillips SM. 2018. Muscle Androgen Receptor Content but Not Systemic Hormones Is Associated With Resistance Training-Induced Skeletal Muscle Hypertrophy in Healthy, Young Men. *Front Physiol.* 9:1373.
- Nielsen TL, Hagen C, Wraae K, Bathum L, Larsen R, Brixen K, Andersen M. 2010. The impact of the CAG repeat polymorphism of the androgen receptor gene on muscle and adipose tissues in 20-29-year-old Danish men: Odense Androgen Study. *Eur J Endocrinol.* 162(4):795-804.
- Norman AW, Mizwicki MT, Norman DP. 2004. Steroid-hormone rapid actions, membrane receptors and a conformational ensemble model. *Nat Rev Drug Discov.* 3(1):27-41.
- Palazzolo I, Gliozzi A, Rusmini P, Sau D, Crippa V, Simonini F, Onesto E, Bolzoni E, Poletti A. 2008. The role of the polyglutamine tract in androgen receptor. *J Steroid Biochem Mol Biol.* 108(3-5):245-253.
- Ponce Gonzalez JG, Guadalupe-Grau A, Rodriguez-Gonzalez FG, Torres-Peralta R, Morales-Alamo D, Rodriguez-Garcia L, Diaz-Chico BN, Lopez Calbet JA, Dorado C. 2017. Androgen receptor gene polymorphisms and maximal fat oxidation in healthy men. A longitudinal study. *Nutr Hosp.* 34(5):1089-1098.

- Ryan CP, Georgiev AV, McDade TW, Gettler LT, Eisenberg DTA, Rzhetskaya M, Agustin SS, Hayes MG, Kuzawa CW. 2017a. Androgen receptor polyglutamine repeat length (AR-CAGn) modulates the effect of testosterone on androgen-associated somatic traits in Filipino young adult men. *Am J Phys Anthropol.* 163(2):317-327.
- Ryan CP, McDade TW, Gettler LT, Eisenberg DT, Rzhetskaya M, Hayes MG, Kuzawa CW. 2017b. Androgen receptor CAG repeat polymorphism and hypothalamic-pituitary-gonadal function in Filipino young adult males. *Am J Hum Biol.* 29(1):e22897.
- Sculthorpe N, Solomon AM, Sinanan AC, Bouloux PM, Grace F, Lewis MP. 2012. Androgens affect myogenesis in vitro and increase local IGF-1 expression. *Med Sci Sports Exerc.* 44(4):610-615.
- Semsarian C, Wu MJ, Ju YK, Marciniak T, Yeoh T, Allen DG, Harvey RP, Graham RM. 1999. Skeletal muscle hypertrophy is mediated by a Ca²⁺-dependent calcineurin signalling pathway. *Nature.* 400(6744):576-581.
- Sheppard RL, Spangenburg EE, Chin ER, Roth SM. 2011. Androgen receptor polyglutamine repeat length affects receptor activity and C2C12 cell development. *Physiol Genomics.* 43(20):1135-1143.
- Simanainen U, Brogley M, Gao YR, Jimenez M, Harwood DT, Handelsman DJ, Robins DM. 2011. Length of the human androgen receptor glutamine tract determines androgen sensitivity in vivo. *Mol Cell Endocrinol.* 342(1-2):81-86.
- Skrgatic L, Baldani DP, Cerne JZ, Ferik P, Gersak K. 2012. CAG repeat polymorphism in androgen receptor gene is not directly associated with polycystic ovary syndrome but influences serum testosterone levels. *J Steroid Biochem Mol Biol.* 128(3-5):107-112.
- Spiering BA, Kraemer WJ, Vingren JL, Ratamess NA, Anderson JM, Armstrong LE, Nindl BC, Volek JS, Hakkinen K, Maresh CM. 2009. Elevated endogenous testosterone concentrations potentiate muscle androgen receptor responses to resistance exercise. *J Steroid Biochem Mol Biol.* 114(3-5):195-199.
- Tut TG, Ghadessy FJ, Trifiro MA, Pinsky L, Yong EL. 1997. Long polyglutamine tracts in the androgen receptor are associated with reduced trans-activation, impaired sperm production, and male infertility. *J Clin Endocrinol Metab.* 82(11):3777-3782.
- van Royen ME, van Cappellen WA, de Vos C, Houtsmuller AB, Trapman J. 2012. Stepwise androgen receptor dimerization. *J Cell Sci.* 125(Pt 8):1970-1979.
- Walsh S, Zmuda JM, Cauley JA, Shea PR, Metter EJ, Hurley BF, Ferrell RE, Roth SM. 2005. Androgen receptor CAG repeat polymorphism is associated with fat-free mass in men. *J Appl Physiol (1985).* 98(1):132-137.

- Wyce A, Bai Y, Nagpal S, Thompson CC. 2010. Research Resource: The androgen receptor modulates expression of genes with critical roles in muscle development and function. *Mol Endocrinol.* 24(8):1665-1674.
- Yin L, Lu L, Lin X, Wang X. 2020. Crucial role of androgen receptor in resistance and endurance trainings-induced muscle hypertrophy through IGF-1/IGF-1R- PI3K/Akt-mTOR pathway. *Nutr Metab (Lond).* 17:26.
- Zhang T, Liang W, Fang M, Yu J, Ni Y, Li Z. 2013. Association of the CAG repeat polymorphisms in androgen receptor gene with polycystic ovary syndrome: a systemic review and meta-analysis. *Gene.* 524(2):161-167.

Table 1. Basic characteristics of bodybuilders

Characteristics	Males <i>n</i> = 40	Females <i>n</i> = 21
Age, years	30.5 ± 8.6	30.0 ± 8.0
Height, cm	176.3 ± 6.8	165.4 ± 4.8
Weight, kg	91.2 ± 14.6	60.7 ± 6.7
BMI, kg/m ²	29.3 ± 3.7	22.2 ± 2.0
Years of resistance training	11.5 ± 6.7	7.9 ± 4.1

Data are expressed as mean (SD).

Table 2. Anthropometric and strength measurements of male ($n = 40$) and female ($n = 21$) bodybuilders with different AR (CAG) $_n$ alleles

Gender	Traits	AR (CAG) $_n$ alleles		P -value	
		Short (< 21)	Long (\geq 21)	Unadjusted	Adjusted
Male bodybuilders	Height, cm	176.7 (5.5)	175.9 (8.0)	0.691	0.621
	Weight, kg	88.6 (10.3)	93.7 (17.8)	0.271	0.194
	BMI, kg/m ²	28.4 (3.1)	30.1 (4.1)	0.131	0.048
	Absolute muscle mass, kg	48.3 (11.7)	56.1 (11.2)	0.046	0.059
	Mid-upper arm, cm	37.7 (4.4)	40.0 (4.4)	0.081	0.036
	Thigh, cm	62.2 (4.2)	65.5 (5.9)	0.056	0.071
	Bench press, kg	156.3 (34.6)	187.8 (40.3)	0.018	0.038
	Squat, kg	212.4 (42.1)	240.0 (44.6)	0.076	0.182
Female bodybuilders	Height, cm	164.8 (4.0)	166.0 (5.6)	0.567	0.359
	Weight, kg	57.4 (4.2)	63.8 (7.3)	0.027	0.034
	BMI, kg/m ²	21.2 (1.6)	23.1 (1.9)	0.020	0.023
	Absolute muscle mass, kg	29.1 (2.8)	32.8 (5.9)	0.085	0.145
	Mid-upper arm, cm	27.1 (1.5)	28.8 (2.9)	0.116	0.163
	Thigh, cm	54.3 (2.4)	56.9 (3.1)	0.043	0.083
	Bench press, kg	57.5 (12.9)	90.6 (26.0)	0.044	0.243
	Squat, kg	68.0 (7.6)	126.7 (33.3)	0.004	0.021

Data are expressed as mean (SD). Associations between AR (CAG) $_n$ alleles and anthropometric measurements were adjusted for age and training experience. Associations between AR (CAG) $_n$ alleles and strength measurements were adjusted for age, training experience and weight.

Table 3. Allele frequency of the *AR* (CAG)_n polymorphism in male athletes and controls

Group	<i>n</i>	<i>AR</i> (CAG)_n alleles (%)		<i>P</i>-value
		Short (< 21)	Long (≥21)	
Sprinters (running)	35	28.6	71.4	0.232
Weightlifters	38	18.4	81.6	0.015
Elite bodybuilders	11	18.2	81.8	0.211
All athletes	84	22.6	77.4	0.0076
Controls	186	39.2	60.8	—

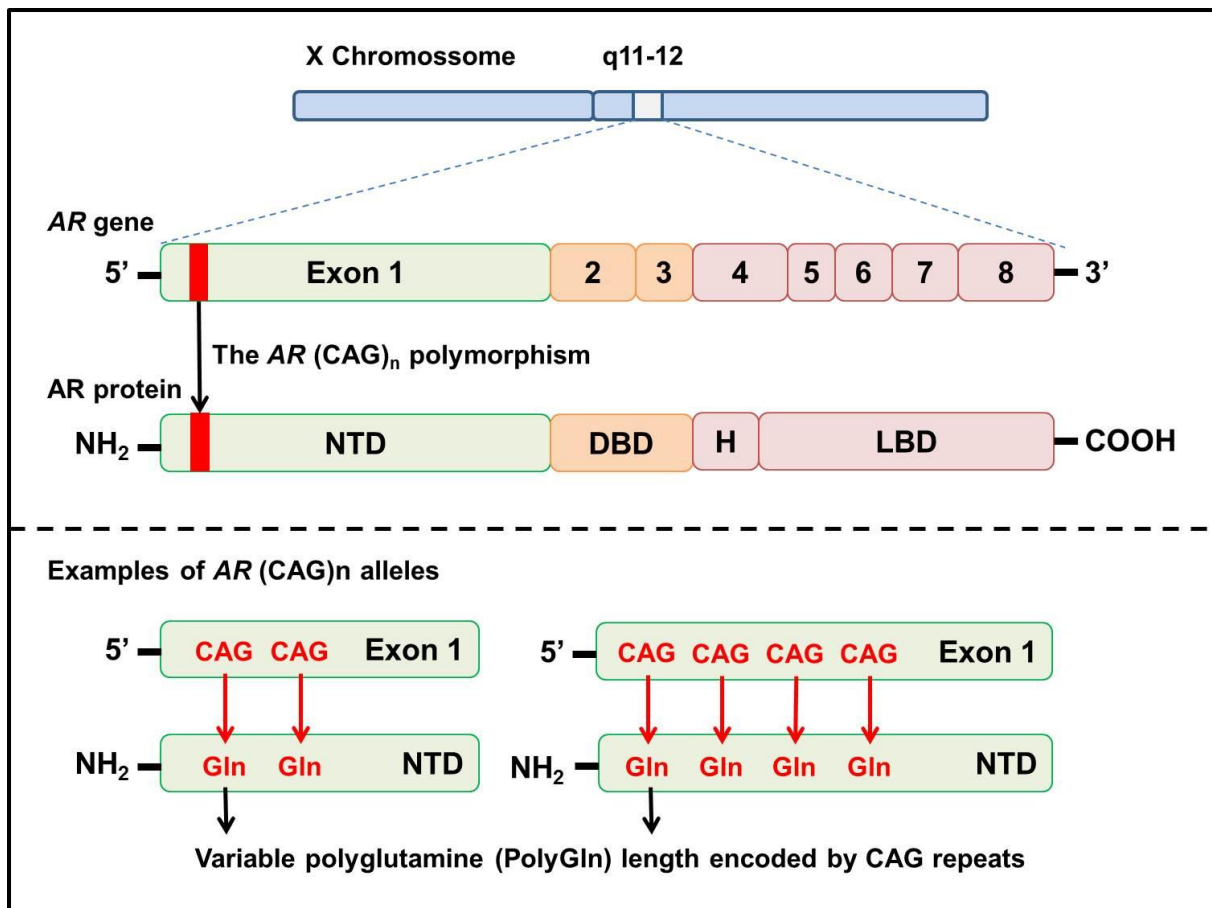


Figure 1. Schematic representation of the AR gene (CAG)_n polymorphism. NTD: NH₂-terminal domain, DBD: DNA-binding domain, H: Hinge region, LBD: Ligand-binding domain.