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REVIEW

# The CAG repeat polymorphism within the androgen receptor gene and maleness[1]

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## Summary

The androgen testosterone and its metabolite dihydrotestosterone exert their effects on gene expression and thus effect maleness via the androgen receptor (AR). A diverse range of clinical conditions starting with complete androgen insensitivity has been correlated with mutations in the AR. Subtle modulations of the transcriptional activity induced by the AR have also been observed and frequently assigned to a polyglutamine stretch of variable length within the N-terminal domain of the receptor. This stretch is encoded by a variable number of CAG triplets in exon 1 of the AR gene located on the X chromosome. First observations of pathologically elongated AR CAG repeats in patients with X-linked spino-bulbar muscular atrophy showing marked hypoandrogenic traits were supplemented by partially conflicting findings of statistical significance also within the normal range of CAG repeat length: an involvement of prostate tissue, spermatogenesis, bone density, hair growth, cardiovascular risk factors and psychological factors has been demonstrated. The highly polymorphic nature of glutamine residues within the AR protein implies a subtle gradation of androgenicity among individuals within an environment of normal testosterone levels providing relevant ligand binding to ARs. This modulation of androgen effects may be small but continuously present during a man's lifetime and, hence, exerts effects that are measurable in many tissues as various degrees of androgenicity and represents a relevant effector of maleness. It remains to be elucidated whether these insights are important enough to become part of individually useful laboratory assessments.

**Keywords:** androgen receptor, CAG repeat polymorphism, testosterone

## Introduction

The variations between genders are mediated by sex hormones, a process starting in utero. As complete insensitivity to androgens leads to a female phenotype (Quigley *et al.*, 1995; Meschede *et al.*, 2000), maleness could be described as the sublimate of gender difference. Testosterone (T) and its metabolite dihydrotestosterone (DHT) exert their effects on gene expression via the androgen receptor (AR). A diverse range of clinical conditions starting with complete androgen insensitivity (CAIS) has been correlated with mutations in the AR (Quigley *et al.*, 1995; Meschede *et al.*, 2000). Subtle modulations of the transcriptional activity induced by the AR have also been observed and frequently assigned to a polyglutamine stretch of variable length within the N-terminal domain of the receptor. This stretch is encoded by a variable number of CAG triplets in exon 1 of the AR gene being located on the X chromosome (Fig. 1). First observations of pathologically elongated AR CAG repeats in patients with X-linked spino-bulbar muscular

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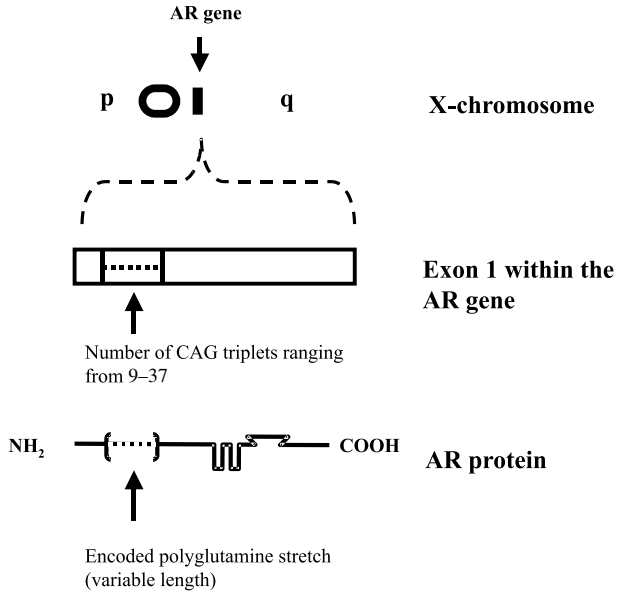


**Figure 1.** Display of the X chromosome with the androgen receptor (AR) gene at q11–12. Exon 1 contains a variable number CAG repeats which encodes for a polyglutamine stretch of variable length in the receptor protein. The number of CAG repeats or length of polyglutamine residues is inversely correlated with the transcriptional activity of androgen target genes.

atrophy (XSBMA) showing marked hypoandrogenic traits (La Spada *et al*., 1991) were supplemented by partially conflicting findings of clinical significance also within the normal range of CAG repeat length. An involvement of prostate cancer risk, spermatogenesis, bone density, hair growth, cardiovascular risk factors and psychological implications has been demonstrated. In this article, these reports will be reviewed with regard to maleness; nevertheless, relevant information concerning effects on female (patho)physiology exists as well.

## Definition of maleness and androgenicity

From the viewpoint of endocrinology, maleness can be described as the phenotypical correlate of androgen effects in humans, because their complete absence during foetal development will lead to a female phenotype, as encountered in testicular feminization of individuals with a 46, XY karyotype. The degree of maleness could then be designated as androgenicity. A male phenotype under-exposed to androgens varies with the time point of onset, the hallmarks being foetal development and puberty. As T and DHT are hormones present in the circulation and ARs are expressed in almost every tissue, androgenicity is exerted nearly ubiquitously. Particularly affected are tissues with a relative abundance of androgen receptors: prostate, testis, bones, larynx, haematopoietic cells, some types of hair follicles and the brain. The clinical picture of low androgenicity, named hypogonadism, is most striking and well known, especially in these organs (Behre *et al*., 2000; Nieschlag *et al*., 2000). Affected individuals ususally present with a small prostate, reduced spermatogenesis, low bone density, a high voice (when exposed to low androgen levels prepubertally), anaemia, a feminized pattern of secondary hair growth and distinct psychological features such as decreased libido, depression and reduced abilities of spatial cognition. Androgen effects can also be observed in lipid and glucose metabolism, and in fat cell physiology (e.g. Oh *et al*., 2002). The T levels within the normal range will more or less saturate present androgen receptors and it has been partially demonstrated that androgenic effects will reach a plateau at certain levels, which are probably tissue-specific (Zitzmann *et al*., 2002a, b). Therefore, it can be assumed that within the range of such a plateau of saturation, genetically determined functional differences in androgen receptor activity can be best observed, while in a condition of hypogonadism, androgenicity will be strongly dependent on T and DHT levels. This could also mean that in some patients with T levels at the lower limit of normal (12 nmol/L), symptoms of hypogonadism will occur and not in others, depending on their genetically determined intrinsic AR activity.

## Functionality of the androgen receptor

The AR is an intracellular transcription factor that belongs to the steroid/nuclear receptor superfamily; other members are receptors for oestrogen, adrenal hormones, vitamin D and thyroid hormone (Quigley *et al*., 1995). When the AR is ligand-activated by androgens and translocates to the nucleus, it binds in dimerized form to specific genomic DNA sequences, which are called androgen response elements in the regulatory regions of androgen-dependent genes. Binding of the androgen–AR complex activates or represses the expression of androgen-regulated proteins (Choong & Wilson, 1998). Thus, ARs are key molecular switches controlling the transcription of androgen-dependent proteins from embryogenesis to adulthood. The AR gene is localized on the X chromosome at Xq11–12 (Brown *et al*., 1989), is encoded in eight exons (Lubahn *et al*., 1988) and has, like other members of the steroid receptor superfamily, three main functional domains: the transactivation domain (TAD), the DNA-binding domain and the ligand-binding domain (Mangelsdorf *et al*., 1995). Differences in the AR sequence are characterized mostly by a highly polymorphic trinucleotide repeat (CAG) encoding a polyglutamine stretch in the TAD (Choong & Wilson, 1998). The normal length of CAG repeats is probably 9–37 (e.g. Hsing *et al*., 2000a; Platz *et al*., 2000), expanded numbers of CAG repeats in the AR are observed in the neurological disorder of XBSMA (e.g. La Spada *et al*., 1991). The basal and ligand-induced transactivational activity of the AR is inversely associated with the length of this CAG repeat chain (e.g. Mhatre *et al*., 1993). The modulatory effect on androgen-dependent gene transcription seems to be rather

linear over a range from 0 to 200 repeats as seen in in vitro studies (e.g. Nakajima *et al.*, 1996) and is probably mediated by causing a differential affinity of coactivator proteins to the encoded polyglutamine stretch, such as ARA24 and p160 (Hsiao *et al.*, 1999; Irvine *et al.*, 2000). As these proteins are ubiquitously but nevertheless non-uniformly expressed, the modulatory effect of the CAG repeat chain on AR target genes is most likely not only dependent on androgenic saturation and AR expression, but also varies from tissue to tissue.

## Kennedy syndrome: a pathological expansion of the AR gene CAG repeats

The XSBMA or Kennedy syndrome, is a rare inherited neurodegenerative disease characterized by progressive neuromuscular weakness which results from a loss of motor neurones in the brain stem and spinal cord. Disease onset developing in the third to fifth decades of life is often preceded by muscular cramps on exertion, tremor of the hands and elevated muscle creatine kinase (Kennedy *et al.*, 1968). The initial description of one of the individuals affected with Kennedy syndrome contains information about treatment for gynaecomastia (Kennedy *et al.*, 1968). Subsequent reports emphasized the presence of signs indicating the development of androgen insensitivity in men with XSBMA who manifest varying degrees of gynaecomastia, testicular atrophy, disorders of spermatogenesis, elevated serum gonadotropins and also diabetes mellitus (e.g. Arbizu *et al.*, 1983). The AR was therefore regarded as candidate gene for XSBMA and the expansion of the polyglutamine repeat within the N-terminal region was subsequently recognized as the cause (La Spada *et al.*, 1991). The longer the CAG repeat in the AR gene, the more severe the symptoms of hypoandrogenicity and earlier the onset of the disease is observed (Doyu *et al.*, 1992; Choong & Wilson, 1998; Mariotti *et al.*, 2000). The absence of any neuromuscular deficit or degeneration in patients with CAIS (Quigley *et al.*, 1995) suggests that neurological deficits in XBSMA are not caused by a lack of androgen influence but rather that a neurotoxic effect is associated with the pathologically elongated number of CAG repeats, causing irregular processing of the AR protein and accumulation of end-products (Abdullah *et al.*, 1998).

## Ethnic differences

The normal range of CAG repeats is probably 9–37 following a normal, in some studies slightly skewed distribution towards the higher number of triplets (Edwards *et al.*, 1992; Hsing *et al.*, 2000a; Platz *et al.*, 2000; Kuhlenbäumer *et al.*, 2001). XBSMA symptoms seem to start at 38–40 CAG repeats (Pioro *et al.*, 1994). Within the normal range of the AR polyglutamine stretch, significant ethnic differences have been observed. For healthy men of African descent the mean number of CAG repeats has been described between 18 and 20 (Edwards *et al.*, 1992; Platz *et al.*, 2000) and seems to be even shorter in certain African subpopulations (Kittles *et al.*, 2001). In healthy Caucasians, the mean number of CAG repeats is 21–22 (Edwards *et al.*, 1992; Platz *et al.*, 2000) while in East Asians a mean of 22–23 triplets is found (Hsing *et al.*, 2000a; Platz *et al.*, 2000; van Houten & Gooren, 2000; Wang *et al.*, 2001).

## The prostate

The prostate is an androgen-regulated organ. Although evidence of a significant role of hormones in the aetiology of prostate cancer is strong, it is still rather circumstantial. Withdrawal of T is used to retard the growth of prostate cancer, suggesting that carcinoma cells are at least initially partly dependent on androgens. As androgens play a role in the development of prostate cancer, it is almost never seen in hypogonadotropic patients or those with 5α-reductase deficiency. Thus, while androgens are capable of stimulating the growth of a malignant prostate process, there is no evidence that they can initiate the transformation of benign into malign prostate cells (for review see Frick *et al.*, 1998).

A substantial difference exists in the incidence of clinical prostate cancer between ethnic groups, with African Americans having a 20–30-fold higher incidence than East Asians (Hsing *et al.*, 2000b). Such disparity cannot be explained entirely by screening bias in different populations. Even after multiple adjustments for ethnic and screening differences, a significant contrast in incidence rates between African Americans, Caucasians and Asians is found (Ross *et al.*, 1998; Platz *et al.*, 2000).

It can be assumed that a polymorphism of the AR with the capacity to modulate androgenicity has an influence on the fate of malignant cells in the prostate. Thus, with shorter CAG repeats, earlier onset of the disease would be observed, as well as an association with aggressiveness of the tumour. Investigation of a younger study group would then lead to the supposition of an increased risk to develop prostate cancer. While this would hold true for a specific younger age group, it is likely that the effect cannot be observed when older men are also involved. Stratification for life-style factors and multidimensional matching of controls in a sufficient number of subjects would be ideal in such investigations: this is best met by seven studies (Giovannucci *et al.*, 1997; Stanford *et al.*, 1997; Correa-Cerro *et al.*, 1999; Hsing *et al.*, 2000a; Platz *et al.*, 2000; Beilin *et al.*, 2001; Latil *et al.*, 2001), the first two, however, using the same population. Six investigations saw an independent contribution of the CAG repeat polymorphism to prostate cancer, either to the age of onset or to the general risk of development. Age of the study group, time point and intensity of diagnostic performance varying with the location of the study are likely to influence the result as to whether it is seen as earlier onset

or higher risk; the putative association with disease stage is likely to be influenced by such factors as well. Each triplet may thus account for a 3–14% risk for prostate cancer (Stanford *et al*., 1997). Summarizing, it is likely that the genesis of prostate cancer cells is not induced by androgens, but that stronger androgenicity induced by ARs with shorter polyglutamine stretches contribute to a faster development of these cells that might be seen as earlier onset of or higher risk for prostate cancer, depending on the age of the study group.

Another aspect is the possible relation between benign hyperplasia of the prostate (BPH) and the CAG repeat polymorphism of the AR gene. BPH consists of the overgrowth of the tissue of the transition zone and periurethral area of the prostate, which is histologically defined as epithelial and fibromuscular hyperplasia (Price *et al*., 1990). One factor that modulates androgenic exposure is the cellular level of androgens, particularly DHT. The influence of the CAG repeat polymorphism in modulating such effects has been investigated in several studies. The two largest studies comparing matched healthy controls ($n = 1041$ and 499) and BPH patients ($n = 310$ and 449) found the odds ratio for BPH surgery or an enlarged prostate gland to be 1.92 ($p = 0.0002$) when comparing CAG repeat length of 19 or less with 25 or more. For a six-repeat decrease in CAG repeat length, the odds ratio for moderate or severe urinary obstructive symptoms from an enlarged prostate gland was 3.62 ($p = 0.004$) (Giovannucci *et al*., 1999a, b). Similarly, adenoma size was found to be inversely associated with the number of CAG repeats in 176 patients vs. 41 controls (Mitsumori *et al*., 1999).

## Reproductive functions

Stimulation of Sertoli cells by follicle-stimulating hormone (FSH) is a prerequisite in primate spermatogenesis. T represents an important cofactor that takes positive effect on the supporting function of Sertoli cells. Hence, it can be speculated that the CAG repeat polymorphism within the AR gene has a limited influence on spermatogenesis. Such an effect has been observed as severely impaired spermatogenesis in XSBMA patients (Arbizu *et al*., 1983). The investigation of the putative influence of a polyglutamine stretch within the normal length on sperm production requires a sample of well-selected patients in which significant confounders such as obstructive symptoms as a result of infections or congenital aplasia of the vas deferens (CBAVD) as well as impaired spermatogenesis because of hormone disorders (primary or secondary hypogonadism) or deletions in one of the azoospermia-associated regions of the Y chromosome have been ruled out. Control groups consisting of healthy fertile males should be homogenous in terms of ethnic origin (see above) and it should be considered that also within the cohort of fertile controls, sperm densities below 20 million/mL might occur (Rajpert-De Meyts *et al*., 2002). Unfortunately, a fraction of studies on this subject did not exclude such patients strictly enough. Therefore, it is not surprising that conflicting results emerged when infertile and fertile men were compared with regard to their number of CAG repeats. Some studies reported higher numbers of triplets in infertile men (Tut *et al*., 1997; Dowsing *et al*., 1999; Legius *et al*., 1999; Yoshida *et al*., 1999; Yong *et al*., 2000; Mifsud *et al*., 2001; Patrizio *et al*., 2001; Wallerand *et al*., 2001), some did not (Lundberg-Giwercman *et al*., 1998; Hiort *et al*., 1999; Dadze *et al*., 2000; Sasagawa *et al*., 2000; Van Golde *et al*., 2002; Rajpert-De Meyts *et al*., 2002). In contrast to studies from Europe, a relationship between sperm production and CAG repeat length was more likely to be found in mixed populations of Asian origin.

When evaluations involved only fertile men covering the whole range of normal sperm concentrations, it could be demonstrated that a shorter CAG repeat tract is associated with higher sperm numbers (von Eckardstein *et al*., 2001; Rajpert-De Meyts *et al*., 2002). Nevertheless, these data show a marked variation of sperm density in relation to the AR polymorphism. Hence, spermatogenesis is likely to be influenced by the number of CAG repeats within the normal range, but whether this reaches relevance remains doubtful. The range of sperm concentrations leading to infertility is most likely reached at CAG repeat numbers that are associated with XSBMA. Furthermore, it can be assumed that the proportion of men with longer CAG repeats among infertile patients may, in case of strict selection criteria excluding all known causes of infertility, appear higher than in a control population.

## Cardiovascular risk factors

Testosterone plays an ambiguous role in relation to cardiovascular risk factors. Low T levels may have an adverse effect on body composition and insulin sensitivity (e.g. Zmuda *et al*., 1997) as well as on haemostatic parameters (Zitzmann *et al*., 2002c). Notwithstanding, androgens have a lowering effect on high density lipoprotein (HDL) cholesterol (Whitsel *et al*., 2001) and most reports on the role played by T on vascular endothelial functions demonstrate a negative influence but are partly conflicting and seem to be dependent on endogenous hormone levels and on dosage and administration pathways of external T (Zitzmann *et al*., 2002a). It has been recently shown in 110 healthy younger males aged 20–50 years that the CAG repeat polymorphism of the AR gene significantly reduces T effects on HDL cholesterol levels and flow-mediated dilatation of the brachial artery (representing endothelial function). The longer the polyglutamine stretch in the AR protein, the higher HDL cholesterol levels and arterial vasoreactivity were found (Zitzmann *et al*., 2001a). Corresponding findings were described for patients with XSBMA (Dejager *et al*., 2002). Possibly longer CAG

repeats in the AR gene might act as a cardioprotective factor, but similar to the androgens that activate the AR, the receptor polymorphism may play an ambiguous role in the determination of atherosclerotic risk: it has recently been demonstrated that higher body fat content and insulin resistance are found in men with longer CAG repeats (Zitzmann *et al.*, 2002d). An inclination to develop diabetes mellitus has been described in patients with XSBMA (Arbizu *et al.*, 1983). Hence, adverse or beneficial effects of a longer or shorter CAG repeat chain with regard to cardiovascular risk will most likely depend strongly on cofactors.

## Alopecia

Male pattern baldness is a common, partly heritable, loss of scalp hair and affects up to 80% of males by age 80 years of age. A balding scalp is found in the presence of androgens and expression of the AR in the respective hair follicle and is thus known as androgenetic alopecia (Randall, 1998). It could therefore be assumed that the modulatory influence of the CAG repeat polymorphism within the AR gene sublimates to a variation of androgenetic alopecia. In men with such a condition, significantly shorter CAG repeat residues were described in comparison with controls (Sawaya & Shalita, 1998). In agreement, in 446 men with androgenetic alopecia shorter CAG repeats were found vs. 107 controls. However, statistical significance was only reached after further inclusion of the GGC polymorphism of the AR (Ellis *et al.*, 2001). Thus, the CAG repeat polymorphism is likely to play a role in modulation of androgen influence on male hair pattern, but as statistical significance is weak in a reasonable number of patients because of high interindividual variability, the cosmetic consequence for the individual is questionable.

## Bone density

In healthy men, polymorphisms of the oestrogen receptor have repeatedly been demonstrated to modulate quantity and quality of bone tissue (e.g. Sapir-Koren *et al.*, 2001). This seems to apply to the CAG repeat polymorphism in the AR gene as well: in 110 healthy younger males aged 20–50 years, a high number of CAG repeats was significantly associated with lower bone density (Zitzmann *et al.*, 2001b), a result that is corroborated by a similar finding in perimenopausal women (Sowers *et al.*, 1999). Results in older men are partly conflicting: one report demonstrated a negative association between AR CAG repeat length and bone density at the femoral neck in a group of 508 Caucasian men aged over 65 years (Zmuda *et al.*, 2000a). The same workgroup also observed a more pronounced bone loss at the hip and increased vertebral fracture risk among older men with longer AR CAG repeat length (Zmuda *et al.*, 2000b), while in a group of 273 healthy Belgian men aged 71 and 86 years, no influence of the AR gene polymorphism was seen (Van Pottelbergh *et al.*, 2001). As higher androgenization will lead to a higher peak bone mass (Khosla, 2002), AR polymorphism effects on bone density are likely to be visible among healthy younger males, while the difference could be mitigated by the overall age-dependent bone loss and may no longer be visible in old men, in whom many confounders have exerted influence on bone mass. Thus, the longer the CAG repeat in the AR gene, the lower peak bone density in males will be found, while it is controversial whether this effect will reach clinical significance in terms of higher fracture risk occurring during age-dependent bone loss.

## Conclusion

The highly polymorphic nature of glutamine residues within the AR protein causes a subtle gradation of

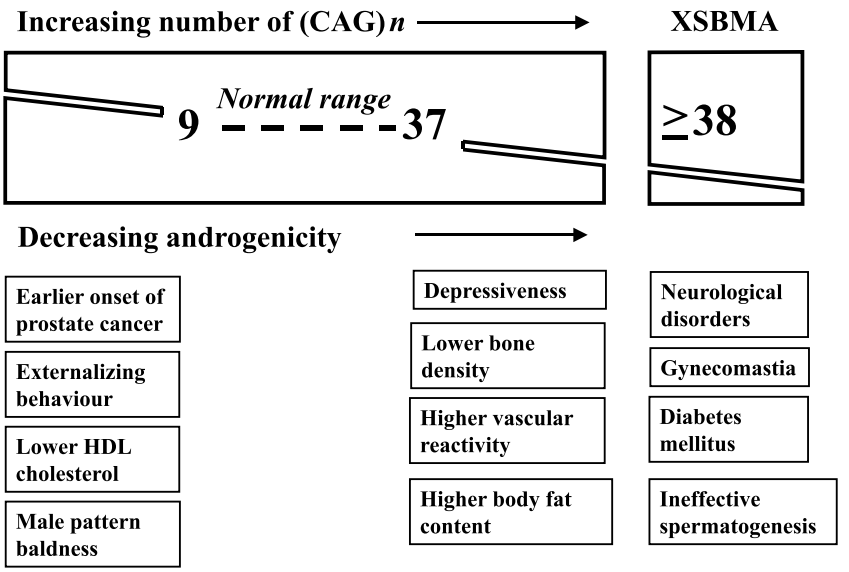


**Figure 2.** The inverse association between the number of CAG repeats in the AR gene and functionality of the AR protein. Longer CAG tracts result in lower transcription of target genes and, thus, lower androgenicity. Expansion of the encoded polyglutamine stretch to beyond probably 38 leads to the neuromuscular disorder X-linked spinal bulbar muscular atrophy (XSBMA), a condition in which defective spermatogenesis and undervirilization are observed. Conversely, low numbers of CAG repeats are associated with increased androgenicity of susceptible tissues.

androgenicity among individuals. This modulation of androgen effects may be small but continuously present during a man's lifetime, thus exerting effects that are measurable in many tissues as various degrees of androgenicity (Fig. 2). It remains to be elucidated whether these insights are important enough to become part of individually useful laboratory assessments. Nevertheless, in interaction with androgens, the CAG repeat polymorphism within the AR gene represents a relevant effector of maleness.

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