Pharmacogenetics of testosterone replacement therapy

Variable phenotypes of androgen insensitivity exist in humans, mainly owing to defective, mutated androgen receptors. A more subtle modulation of androgen effects is related to the CAG repeat polymorphism ([CAG]n) in exon 1 of the androgen receptor gene, *in vitro*, transcription of androgen-dependent target genes is attenuated with increasing length of triplets. As a clinical entity, the CAG repeat polymorphism can relate to variations of androgenicity in (apparently) eugonadal men in various tissues and psychological traits, the longer the (CAG)n, the less prominent the androgen effect when individuals with similar testosterone concentrations are compared. A strictly defined threshold to hypogonadism is likely to be replaced by a continuum spanned by genetics as well as symptom specificity. In addition, effects of externally applied testosterone can be markedly influenced by the (CAG)n and respective pharmacogenetic implications are likely influence indications as well as modalities of testosterone treatment of hypogonadal men.

KEYWORDS: androgen receptor ▪ male hypogonadism ▪ pharmacogenetics ▪ testosterone

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Male hypogonadism is a nosological entity manifesting itself with a variety of symptoms owing to a depletion of testosterone or its attenuated action. For substitution purposes, testosterone preparations have been used for approximately 60 years with interindividually different responses to the substitution of testosterone [1]; a partial explanation for this phenomenon can be found in pharmacogenetic approaches to the androgen receptor (AR). The diagnosis of hypogonadism has always been constrained by strict definitions of thresholds of serum androgen concentrations. New insights in symptom specific thresholds of hypogonadism [2], as well as genetically determined degrees of androgen action [3], are challenging the concept of generally applied simple thresholds [4]. In addition, pharmacogenetically tailored testosterone substitution/application might emerge as an aspect to explore in the future to optimize the balance between clinical benefits and risks of treated hypogonadal men [5].

Physiology of AR

The AR is intracellularly located and structurally related to other steroid hormone receptors. The transcriptional regulation mediated by the AR is a process involving ligand (i.e., androgen) binding and interaction of cofactor, as well as conformational changes of the AR protein, receptor phosphorylation and nuclear trafficking and binding to DNA target regions, and, finally, transcription activation. The human *AR* gene is located on the X-chromosome (Xq11–12) (e.g., [6]), spanning approximately 90 kb, comprising eight exons.

The AR has a modular composition of three major functional domains: a N-terminal domain precedes the DNA-binding domain, followed by the C-terminal ligand-binding region (for review see [7]). Upon entering target cells, androgens will interact with the ligand-binding pocket of the AR, thus initiating an activation cascade that includes change of conformation and nuclear translocation. Prior to binding at target DNA regions, homodimerization of two AR proteins occurs. The resulting homodimer binds to hormone responsive elements, usually consisting of two palindromic sequences within the promoter regions of androgen-regulated genes. Facilitated by chromatin remodelling, a direct interaction with other transcription factors and specific coactivators, repressors and a specific modulation of the assembly of the preinitiation complex is achieved: this results in specific activation or repression of target gene transcription [8].

Not only testosterone binds to the AR, its 5-α-reductase metabolite, dihydrotestosterone, also binds with a much stronger relationship. Estrogenic hormones, progesterone and dehydroepiandrosterone are also able to bind to and activate the AR, but only on a clinically nonrelevant basis [8].

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Clinical implications: mutations & polymorphisms of the androgen receptor

Normal male sexual differentiation is initiated and modulated by the molecular interaction of testosterone and dihydrotestosterone with the AR in respective androgen-responsive target tissues. A wide range of clinical conditions starting with complete androgen insensitivity has been related to mutations in the *AR* gene, the first observations of pathologically elongated *AR* (CAG)n (normal range is 9–37 repeats) were made in patients with X-linked spino-bulbar muscular atrophy: these patients exhibit markedly hypoandrogenic traits [7,9,10]. More subtle modulations of the transcriptional activity induced by the AR have also been observed and can be assigned to a polyglutamine stretch of variable length within the N-terminal domain of the receptor. This stretch is encoded by CAG triplets ([CAG]n) in exon 1 of the *AR* gene (Figure 1).

A 'humanized' mouse model of the *AR* gene (CAG)n directly assessed the functional significance of the *AR* gene (CAG)n: the mouse AR was converted to the human sequence by germline gene targeting, introducing alleles with 12, 21 or 48 (CAG)n. The three humanized AR mouse lines revealed markedly different seminal vesicle weights indicating androgen effects despite similar serum testosterone concentrations. Molecular analysis of AR-dependent target gene expression demonstrated in the prostate probasin, Nkx3.1 and clusterin mRNAs to be modulated by the number of (CAG)n. Crossed with transgenic adenocarcinoma of mouse prostate mice, genotype-dependent differences in prostate cancer prevalence were observed [11]. This confirms findings of *AR* gene (CAG)n expression in human prostate cancer cells [12]. Confirmingly, epidemiological findings in humans researching the incidence of prostate cancer demonstrate an influence of the *AR* gene (CAG)n polymorphism: a meta-analysis described an odds ratio of 1.19 for prostate cancer with decreasing (CAG)n [13]. The modulatory effect on androgen-dependent gene transcription is described to be linear and obviously mediated by a differential affinity of coactivator proteins to the polyglutamine stretch (e.g., ARA24 and p160) [14,15].

Subsequently, findings of clinical significance were reported for the normal range of (CAG)n. An influence of the polymorphism on androgen target tissues such as the prostate, spermatogenesis, bone density, hair, skin, psychological traits, transsexualism and premature adrenarche has been demonstrated [3,16–29]. Especially the feature of depression in men with longer CAG repeats is impressing: it was firstly described by the group around Huhtaniemi in Finland [30] and later confirmed in China [31] and the USA [32]. Also of major health implications is the association of weaker androgen action (longer [CAG]n) with features of the metabolic syndrome [33,34]. The above named complete androgen insensitivity may be, despite a mutation of the androgen receptor at another location, of less severe clinical expression on those patients with an especially active co-activator binding part, that is, rather short (CAG)n [35]. In particular, regarding male fertility, a recent meta-analysis involving 33 reports provides support for an association between increased *AR* gene CAG length and idiopathic male infertility, suggesting that even subtle disruptions in the androgen axis may compromise male fertility [36].

Men presenting with features of hypogonadism may exhibit normal testosterone levels but CAG repeat lengths above the normal average (Europe: 21, Africa: 17, Asia: 23): hence, a CAG repeat length longer than 25 is still considered to be within the normal range, but can already be associated with reduced androgen action and accompanying clinical features, suggesting classical hypogonadism in the case of still normal testosterone concentrations [4].

In Klinefelter patients, who have two *AR* gene alleles due to their 47,XXY karyotype, the shorter CAG repeat allele is preferentially inactive. In this group of patients, CAG repeat length is positively associated with body height, while bone density and the relation of arm span to body height are inversely related to CAG repeat length [37]. The presence of long CAG repeats was seen as predictive for gynecomastia, while shorter CAG repeats were associated with a stable partnership and professions requiring higher standards of education [37]. Also, aspects of puberty and masculinization of younger Klinefelter patients seem to be influenced by this polymorphism, boys who have longer CAG repeats exhibit mitigated androgen effects [38,39]. In the rare syndrome of 46,XX males, the inactivation patterns of *AR* gene alleles in XX males were found to be significantly more skewed than in Klinefelter patients and women. A total of seven of ten heterozygous XX male patients displayed an extreme skewing of more than 80% with no preference toward the shorter or longer *AR* allele. The length of the *AR* gene CAG repeat polymorphism was positively related to traits of hypogonadism [40].

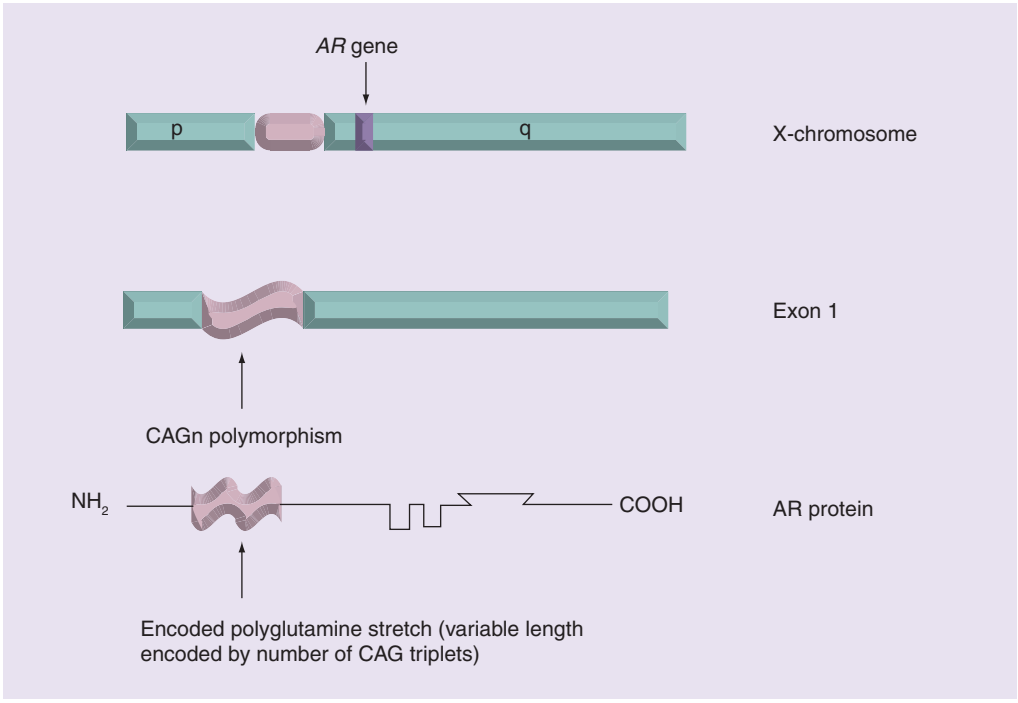

Figure 1. Display of the X-chromosome with the *AR* gene. Exon 1 contains a variable number of CAG repeats encoding a polyglutamine stretch of variable length in the receptor protein. The number of CAG repeats or length of polyglutamine residues is inversely associated with the transcriptional activity of androgen-dependent genes, hence androgen effects in target tissues.
AR: Androgen receptor.

Studies examining the effects of the *AR* gene (CAG)n polymorphism have to consider some aspects for proper description of clinical relevance, for example the involvement of hypogonadal men will distort results owing to lack of sufficient ligand binding, hence, activation of the AR. Similarly, not considering testosterone levels, possibly compensating for the effects of the (CAG)n polymorphism, takes an incomplete approach. When examining the effects of androgens, regression models are required to include both testosterone concentrations and the length of the *AR* gene CAG repeat polymorphism. In this light, a recent study in a large cohort of aging men did not demonstrate a relationship of the *AR* gene CAG repeat polymorphism to the risk of heart disease, possibly due to the fact that serum testosterone concentrations were not considered [41].

The weaker androgen action induced by longer (CAG)n will also affect the feedback mechanism of the hypothalamic–pituitary–gonadal (HPG) axis. In healthy men, longer (CAG)n will usually provoke higher luteinizing hormone (LH) secretion [33,34,42]. This can, in persons with intact and fully responsive Leydig Cell capacity, result in higher concentrations of testosterone and, hence, compensation of weaker androgen action. As a result of higher testosterone concentrations, its aromatization product estradiol will also be present in higher amounts [42]. Such higher estradiol concentrations in these men with intact HPG regulation or feedback mechanisms can even lead to enhanced effects in estrogen-dependent tissues, such as bones [43].

However, investigation of men with an intact HPG axis does not focus on the clinically relevant clientele including men with symptoms of androgen deficiency and disturbances of the HPG axis. Such disorders can be of milder nature, as seen in late-onset hypogonadism [44] or subjects with the metabolic syndrome [45], conditions in which both pituitary function and Leydig cell capacity are impaired. In case of longer (CAG)n, these men will present with inadequate concentrations of LH within the lower normal range and/or testosterone levels that are in the lower normal range, but, nevertheless, will require higher concentrations of testosterone to compensate for their attenuated androgen action. Thus, they are likely to present with features of hypogonadism in the presence of normal testosterone levels [4] (see FIGURE 2). This is the patient group most likely missed for investigation, diagnostics and putative treatment in clinical andrology to date.

In case of 'classical' hypogonadism, for example, the (almost) complete breakdown of the HPG axis due to primary or secondary origin,

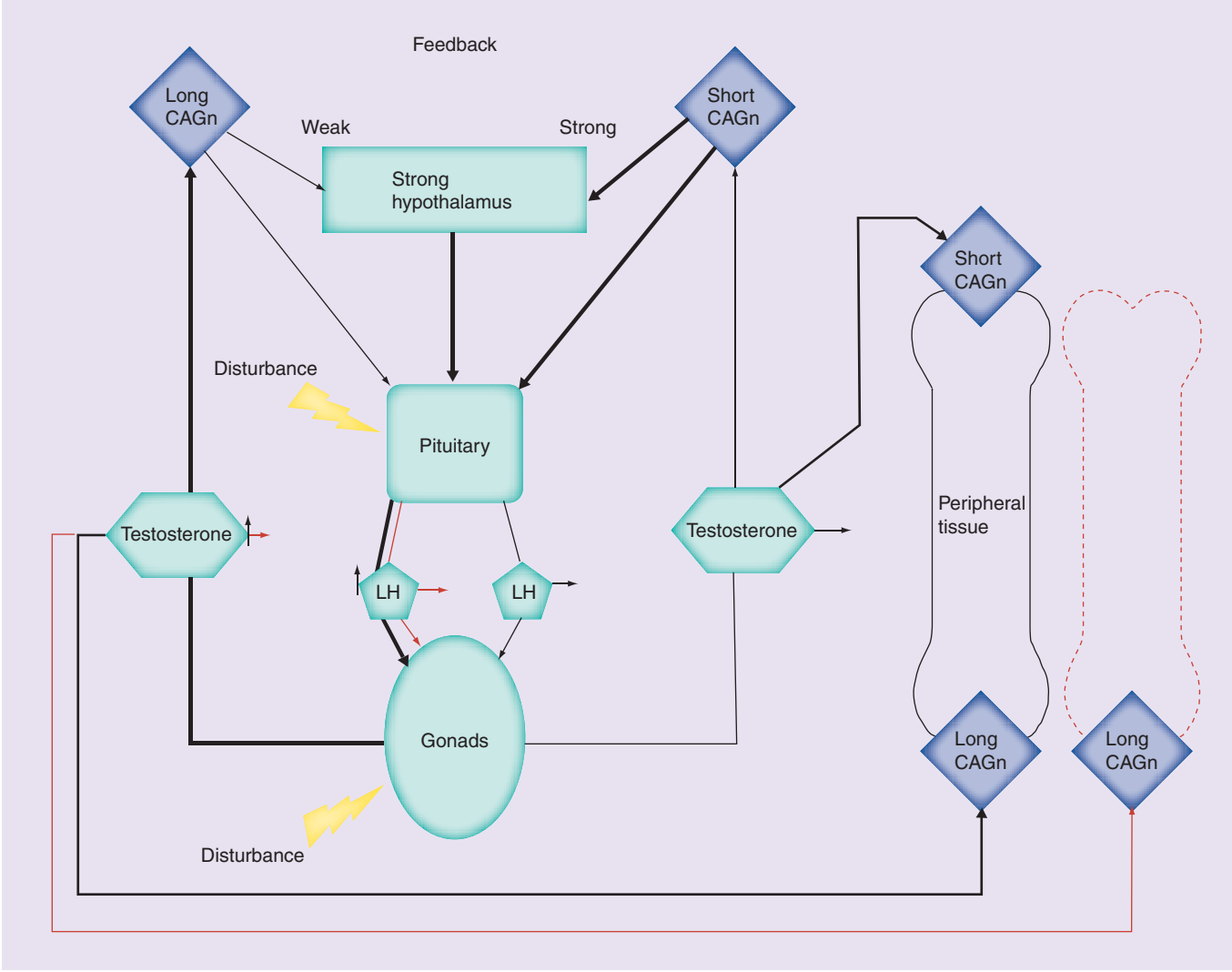

Figure 2. Identification of subjects with mitigated androgen effects due to longer (CAG)n in the androgen receptor gene, hence reduced testosterone-induced transcriptional activity and possible symptoms of hypogonadism. Black: the HPG axis and the feedback mechanism are intact. Weaker androgen action in case of longer (CAG)n is compensated by higher LH concentrations and higher testosterone levels. The peripheral tissue remains intact. Red: any disturbance of the HPG axis or the feedback mechanism (such as [borderline] dysfunction of gonadotropin secretion, Leydig cell capacity) will attenuate the compensation for longer (CAG)n. These persons might still exhibit apparently normal testosterone concentrations, which are, nevertheless, not high enough to maintain peripheral tissue integrity. They are likely to present with symptoms of hypogonadism, albeit presenting androgen levels usually considered normal. Any investigation of the effects of the (CAG)n polymorphism of the androgen receptor gene has to focus on these men as they provide a large patient group possibly escaping attention. Evaluation of men with an intact HPG feedback mechanism conceals this clinical aspect. The same applies for the situation of complete breakdown of the HPG axis (classical hypogonadism) requiring androgen substitution. Providing low normal testosterone levels by external medication is likely to be not sufficient in cases of long (CAG)n.
HPG: hypothalamic–pituitary–gonadal; LH: Luteinizing hormone.

testosterone levels are low and patients require substitution. The findings in men with intact HPG axes demonstrate that persons with longer (CAG)n require higher testosterone concentrations for normal androgen action in comparison with men with shorter (CAG)n [42]. Thus, hypogonadal persons with longer (CAG)n will then, as do their healthy counterparts, need higher testosterone levels (and, hence, higher testosterone doses) to compensate for mitigated androgen action. Correspondingly, persons with rather short (CAG)n will need lower doses of testosterone substitution in case of hypogonadism. This is exactly the subject of pharmacogenetic tailoring of testosterone substitution that will be elucidated below.

Pharmacogenetics of testosterone therapy

Considering the cross-sectional studies performed in eugonadal men, testosterone therapy in hypogonadal men should have a differential

impact on androgen target tissues, depending on the number of *AR* gene (CAG)n. Indeed, a longitudinal pharmacogentic study in 131 hypogonadal men examined prostate volume before and under androgen substitution demonstrated such an effect. The initial prostate size of the hypogonadal patients was dependent on age and baseline testosterone levels, but not (CAG)n length, as sufficient ligand binding to the AR was not present. When testosterone levels were elevated by substitution and, hence, a substrate for the AR was provided, prostate size was augmented markedly. Absolute prostate size as well as prostate growth per year during externally controlled testosterone levels were strongly dependent on (CAG)n length. Treatment effects were mitigated in longer (CAG)n and men with short (CAG)n exhibited augmented prostate growth [46].

Correspondingly, stimulation of erythropoeisis during testosterone application, was modulated by the *AR* gene (CAG)n polymorphism in 66 men under long-term treatment with intramuscularly injected testosterone undecanoate. Nadir levels of testosterone, hence, injection intervals, could be tailored according to (CAG)n length. Those patients with longer (CAG)n required more frequent injections, while those with shorter (CAG)n could be administered their testosterone injections in longer intervals, in order not to increase hematocrit into the pathological range [5].

In 48 hypogonadal Klinefelter patients, baseline concentrations of LH as well as hemoglobin and also prostate size were not related to the (CAG)n polymorphism. When testosterone levels were reconstituted by substitution, suppression of LH concentrations, elevation of hemoglobin concentrations and prostate growth were seen more profoundly in men with shorter (CAG)n [37]. Another approach concerning pharmacogenetic influences of the *AR* gene (CAG)n polymorphism was made in hormonal male contraception trials. Sperm counts were more easily suppressed by various pharmacological regimens in men with longer (CAG)n as spermatogenesis is partially dependent on intratesticular androgen activity: Sertoli cells express the AR and are, partially, dependent on testosterone supply. However, a pharmacogenetic effect was only observed in a subgroup with residual gonadotropin activity, causing stimulatory effects on Leydig cells (by residual LH) to produce testosterone, which can then bind to the Sertoli cell ARs (which were stimulated by residual FSH), thus making differences caused by the CAG polymorphism visible [47]. This observation was recently confirmed in a large trial in China [48]. When all subjects of such trials are involved in a respective analysis and the distinction in regard to residual gonadotropin secretion is not made, the difference between individuals with long or short CAG repeats cannot be observed, as Sertoli

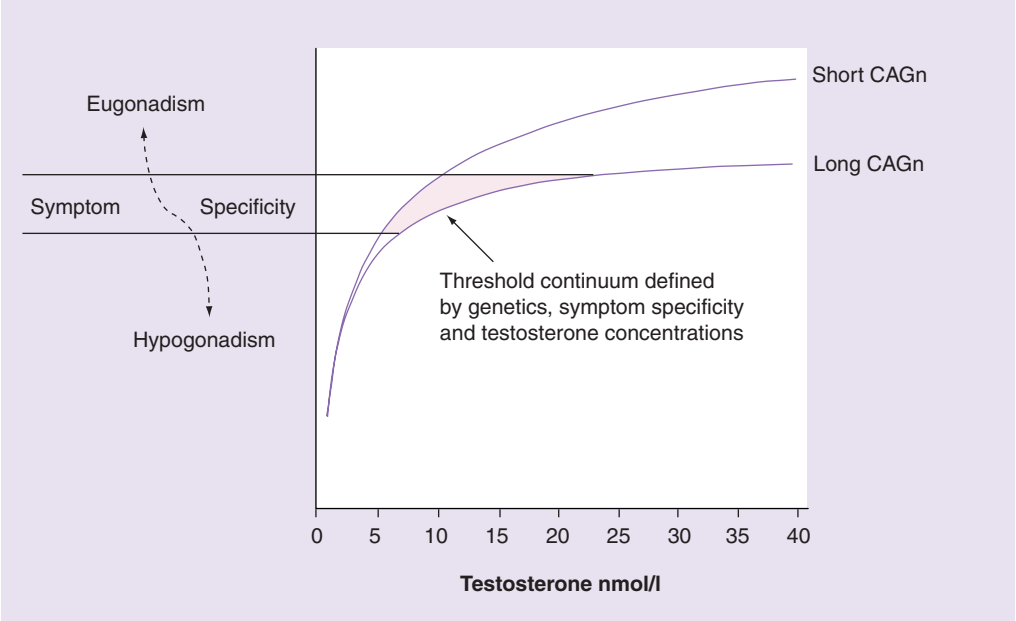

Figure 3. Modulation of androgen effects. Originating in symptom specificity and genetically determined androgen action, no clear-cut threshold for testosterone concentrations exists between eugonadism and hypogonadism. The clinically relevant threshold to hypogonadism is, rather, a continuum (pink), which is individually defined and is spanned by testosterone levels, genetic background and the respective symptom.

cell ARs will not be activated in persons lacking LH/FSH and, hence, intratesticular testosterone action [49].

Another aspect of pharmacogenetic influence of the (CAG)n polymorphism is the susceptibility to the treatment of androgen withdrawal in adverse conditions caused by androgens. Persons with short (CAG)n are used to high androgen input into their pathological entity. One example is the treatment of male pattern baldness with finasteride. Men with shorter (CAG)n tracts respond significantly better to this treatment than men with longer (CAG)n [50]. Also the treatment of postmenarchal androgen-excess in girls, using flutamide, is more effective in those patients with short (CAG)n [51].

Discussion: a hypothetical model of androgen action

Testosterone concentrations within the normal range more or less saturate ARs and it has been shown that androgen effects reach a plateau at certain levels, which are probably tissue- or symptom-specific [52]. Just recently, a saturation model for prostate tissue in relation to testosterone concentrations has been propagated [53]. Marked increments of testosterone-caused effects are only seen beyond the normal range when highly supraphysiological levels, such as in doping, are reached. Hence, it can be argued that within the range of normal eugonadal testosterone concentrations, which are desired to be achieved by substitution therapy of hypogonadal patients, genetically determined functional differences in AR activity can be observed and will be of significance, while in a condition of hypogonadism, androgenicity will rather depend on androgen levels as testosterone binds to ARs and will augment androgen effects until a saturation level is reached (Figure 3).

Upon testosterone substitution of hypogonadal men, effects of the androgen are likely to be induced by the increment of androgen levels from the hypogonadal into the eugonadal range, as well as through modulation mediated by the *AR* gene CAG repeat polymorphism, the latter mainly within the eugonadal range, which is achieved during sufficient therapeutic approaches; the AR needs a normal amount of substrate (testosterone) to exhibit the effects of the (CAG)n polymorphism.

Conclusion

In summary, a confined and universal threshold for testosterone levels to hypogonadism does not exist, but, instead, individual thresholds of testosterone levels according to the length of the CAG repeat polymorphism are likely to be of clinical relevance. The model of Figure 3 explains why androgen effects are found when comparing hypogonadal and eugonadal men, but can often not be described for various testosterone levels within the eugonadal range. Also, tissue- or symptom-specific thresholds of testosterone concentrations as well as testosterone levels themselves contribute to span a continuum between eugondal and hypogonadism respecting individuality rather than a universal threshold.

Especially in regard to late-onset hypogonadism or the metabolic syndrome, clinical entities that are related to advancing age of men or obesity causing a deterioration of the HPG axis and, hence, disturbance of feedback mechanisms that can compensate for long (CAG)n, these aspects might play a role in the decision whether to start testosterone replacement therapy or not.

In the future, substitution therapy could be individually tailored by the *AR* gene (CAG)n polymorphism: men with shorter repeats may require lower doses of testosterone while men with longer repeats could be in need of higher doses of androgens. Correspondingly, men with longer repeat tracts may need substitution therapy at baseline testosterone concentrations, which are still considered normal for the total population. Future pharmacogenetic studies will be required to find clinically robust answers.

It should be mentioned that many other factors are most likely to be influencing testosterone action, be it the endogenous hormone or exongenously administered androgens. Enough genetic information is not available to substantially review the possible effects of other androgen receptor polymorphisms, enzymes involved in androgen metabolism (5-α-reductase, aromatase), protein binding and elimination (conjugation), as well as differences in co-activator and co-repressor activities, most likely tissue-specific. The CAG repeat polymorphism cannot be the only determining factor in androgen sensitivity.

Nonlinear calculation models according to Figure 3 involving testosterone concentrations and the length of the AR (CAG)n may be useful to assess the individually needed testosterone dose to achieve a certain desired androgen-related action or avoid an adverse side effect, such as elevated hematocrit (see Figure 4 for a summary). These insights require further research within the frameset of prospective studies as to this time point only semiquantative statements can be made in terms of testosterone doses needed

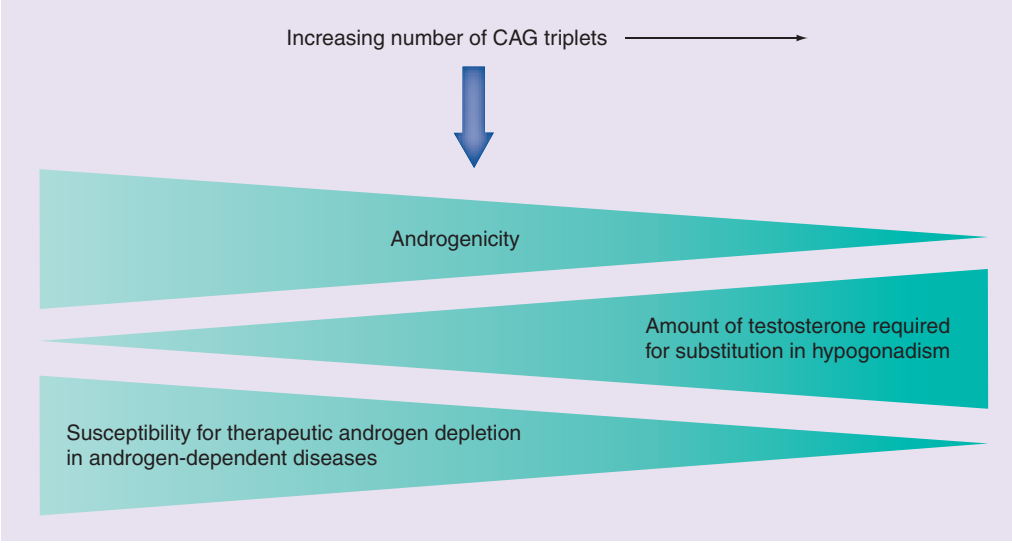

Figure 4. Summary of the clinical relevance of the pharmacogenomically active (CAG)n polymorphism of the androgen receptor gene.

in hypogonadal men with different CAG repeat length [5]. Future calculations might be possible to state the required dose of testosterone in relation to the AR polyglutamine stretch. The different doses that are assumed according to the cross-sectional literature are most likely to be of clinical relevance and not marginal.

The level of knowledge required to include the *AR* gene CAG repeat polymorphism into routine andrological assessments is not sufficient, but it has become a clinically and scientifically worthy concept to determine the CAG repeat length in subjects with normal testosterone levels and unexplained features of hypoandrogenism. Concerning the dose adaption of testosterone to the polymorphism in androgen substitution, independent verification of the above mentioned findings is still required before giving general recommendations.

Future perspective

This is a speculative viewpoint stating that determination of the *AR* gene (CAG)n length will play a clinically robust role in diagnosis and treatment of hypogonadal men within 5–10 years, both for adaption of testosterone levels to the clinical features as well as the dose required for proper androgen substitution.

Review criteria

PubMed and Medline were searched for papers published from January 1990 to April 2009, under the terms 'male hypogonadism', 'testosterone' and 'CAG repeat polymorphism'. Of the citations identified by the searches, papers were selected on the basis of peer-reviewed original articles and, if suitable, controlled clinical trials or larger epidemiological studies. Only papers published in English have been cited in this review.

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Executive summary

- The (CAG)n polymorphism in exon 1 of the androgen receptor gene modulates androgen effects.
- Transcription of androgen-dependent target genes is attenuated with increasing length of these triplet residues.
- The (CAG)n polymorphism causes significant modulations of androgenicity in healthy eugonadal men in various tissues and psychological traits: the longer the repeat tracts, the less pronounced the androgen effect is. To this end, individuals with similar testosterone concentrations have to be compared.
- Men with a normal hypothalamic–pituitary–gonadal axis are likely to present with higher testosterone concentrations in the presence of longer *AR* gene CAG repeats, thus compensating weaker receptor activity.
- Effects of testosterone supplementation in hypogonadism are markedly influenced by the number of (CAG)n.
- The pharmacogenetic implications of the (CAG)n polymorphism of the *AR* gene are likely to play a significant role in future testosterone treatment of hypogonadal men: initiation thresholds for testosterone treatment as well as androgen dosage can be tailored accordingly.

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