Throughout the human genome there are trinucleotide repeat sequences susceptible to either expansion or contraction during replication, giving rise to length polymorphisms in the general population. The polymorphic CAG repeat, which encodes an uninterrupted polyglutamine (polyQ) tract in the N-terminal transactivation domain of the androgen receptor (AR), is the most extensively studied genetic variant in individuals with disorders of the male reproductive system.

Despite an impressive number of studies, the pathogenetic role of this polymorphism and its clinical relevance are still a matter of debate. Although a recent meta-analysis of 33 publications (1) supports a pathogenetic role for longer polyQ length in male infertility, the authors conclude their work stating that there is a need for new, well-designed studies (1). In fact, available data do not allow us to establish what range of AR-CAG repeat lengths predisposes impaired sperm production or to estimate the entity of the associated risk (1). Similar to other genetic variants, the literature related to CAG repeats suffers from an abundance in conflicting case-control association studies and a paucity of functional data (2). There are several plausible explanations for these apparent controversies, mostly related to: 1) poor study design (inappropriate selection of patients and controls, particularly with respect to their phenotype and their ethnic/geographic origin, and underpowered size of the study population); and 2) intrinsic complexity of the interaction between the AR and its endogenous/environmental ligands. An additional intricacy derives from the presence of another polymorphic trinucleotide repeat, (GGN)n, in the first exon of the AR gene, which may modulate the functional effect of the CAG repeat length, stressing the need for a combined analysis of the two AR polymorphisms (3, 4).

It is commonly accepted that the length of the polyQ tract influences the transactivation capacity of the receptor in an inverse manner; that is, the longer the tract, the lower the activity. To support this hypothesis, a clear negative impact on AR activity is documented in relationship with pathological expansions of the repeat length (40 or more), known as the Kennedy syndrome (5). This syndrome is characterized by spinobulbar muscular atrophy and hypoandrogenism due to partial androgen insensitivity. On the other hand, controversies still exist about the effect of variations in polyQ within the normal polymorphic range. The normal distribution of the (CAG)n is reported as 6–39 repeats, with a median of 21–22 in White Caucasian, 19–20 in African-American, 22–23 in Asian, and 23 in Hispanic populations. Clinical observations showing a linear correlation between testosterone level and CAG repeat length support the notion of a functional effect of the polymorphism within the normal range. In fact, increased circulating testosterone and estradiol levels in men with a higher number of CAG repeats can be considered as a compensatory mechanism aimed to overcome the weaker AR activity (6, 7). However, such a linear correlation has not been clearly demonstrated by in vitro experiments. The first two functional studies reported that the longest tract (Q31) displayed lower activity when compared with the shortest one (Q15). However, no significant differences were observed by comparing these two types of alleles to an intermediate number of CAG repeats (20 or 24) (8, 9). Quite strikingly, two recent articles provided evidence for the lack of a stepwise reduction in activity with increasing CAG length across the polymorphic range.
range (10, 11). The reporter gene assay with three different CAG lengths (16, 22, and 28) has indeed shown the highest AR activity in the presence of 22 CAG repeats (10). The other study, performed in a human prostate tumor cell model, has provided mechanistic insights into how both increased and decreased polyQ allele length may negatively affect receptor function (11). This study has revealed a critical polyglutamine size (Q16–Q29) for optimal androgen-induced AR signaling, which corresponds to 91–99% of AR alleles within different ethnic groups. These novel in vitro findings have introduced a new concept for the analysis of AR-CAG repeat length in relationship to AR-related diseases, indicating that linear regression models are likely to be inappropriate.

In this issue of the JCEM, Davis-Dao et al. (12) provide strong evidence for the need of a radical improvement in the study design used to establish the relationship between CAG repeat length and disease phenotypes. The study has focused on cryptorchidism, a well-known androgen-dependent disease. Both clinical evidence (cryptorchidism in subjects affected by androgen resistance) and animal models clearly demonstrate that testosterone is one of the major hormonal regulators of testis descent (13). Therefore, it is expected that variations in the transactivation activity of AR, especially a longer polyQ tract, may be responsible for nonsyndromic testicular maldescent. Despite this prediction, the literature has unanimously dismissed long CAG repeat length as a risk factor for cryptorchidism (3, 4, 14–16). Now, 5 yr after the last published study on this topic, Davis-Dao et al. (12) adopt an innovative study design and a distinct analytical model. For the first time, patient recruitment is performed in a clinical context—boys undergoing orchidopexy—which allows the precise and unequivocal definition of the cryptorchid phenotype and of comorbidities. All previous studies have included adult men with a history of cryptorchidism, and thus considerable difficulties were encountered in providing a rigorous phenotypic characterization. Importantly, in all previous investigations, patients with associated inguinal hernia were systematically excluded or not recorded. In the study of Davis-Dao et al. (12), these cases belong to the most relevant subgroup among which CAG repeat length seems to be of clinical relevance. Another peculiarity concerns the control group, which, instead of unaffected unrelated subjects, refers to the second untransmitted maternal CAG allele. The comparison of cases’ alleles to their mothers’ second not-transmitted alleles is based on the transmission/disequilibrium test for an X-linked marker in males and allows avoidance of population stratification that can be an important bias in admixed populations (17).

The major finding of the study is the unexpected association between shorter CAG repeats (CAG ≤ 19 vs. CAG ≥ 20) and cryptorchidism risk, which was restricted to those cases presenting comorbidities (especially inguinal hernia). Among 127 patients, 64 have shown inguinal hernia as the sole comorbidity, whereas seven had inguinal hernia with other comorbidities (morbid obesity, hypopadias, micropenis, etc.). When considering all cryptorchid cases with any type of comorbidities (n = 83), a significant “protective” role of CAG repeats of ≥ 20 was identified with an odds ratio of 0.35 (95% confidence interval, 0.16–0.78). This association was even stronger in the subgroup of subjects with bilateral cryptorchidism [CAG ≤ 19 vs. CAG ≥ 20, odds ratio = 0.09 (95% confidence interval, 0.010–0.78)]. However, due to the relatively small sample size of this subgroup (n = 25), this finding needs to be interpreted with caution. The intriguing clinical observation showing an association between the shorter polyQ tract (considered the more active receptor) and cryptorchidism with comorbidities highlights the complexity of this issue.

First of all, these results do not seem to fit with any of the two predicted AR-CAG tract models based on the classical genomic pathway: 1) the inverse relationship between CAG number and AR activity; and 2) the “optimal range” hypothesis predicting an altered function for receptors with both shorter and longer polyQ tract with respect to the functionally critical range. To explain the apparent paradox of the observed association between a more active AR and the anomaly of an androgen-dependent process, Davis-Dao et al. (12) speculate on an indirect effect of polyQ length via alternative “nongenomic” signaling pathways. According to their hypothesis, the reduced testosterone level—typically observed in men with shorter polyQ—may lead to a suboptimal signaling of a hypothetical nongenomic pathway involved in testis descent. This hypothesis appears to be particularly attractive in light of those studies demonstrating that cell growth and antiapoptotic actions of androgens could be dissociated from the transcriptional activity of the AR. Several authors have reported that both MAPK and PI3K/Akt pathways can be activated by the AR in cancer cell lines, thus promoting cell proliferation as a consequence of rapid “nongenomic action” (for review, see Refs. 18 and 19). The most recent evidence suggests that the gubernaculum actually acquires specific growth properties similar to an embryonic limb bud (20). The primary hormone regulating transabdominal descent is insulin-like hormone 3 and it is responsible for the “swelling reaction” of the gubernaculum, whereas the second phase is highly dependent on testosterone action. However, recent experiments in rodents indicate an intimate interplay between the two hor-
mone during the entire process of testis descent (for review, see Ref. 13). The swelling reaction anchors the testis to the future inguinal canal. During the second, inguino-scrotal phase of descent, the gubernaculum changes from a relatively inert, static structure ending in the inguinal muscles into an elongating, migrating organ. It has been proposed that androgens trigger the ligamentous gubernaculum to become an outgrowth like a limb bud, although the underlying mechanism remains unknown (21). In humans, the role of an indirect androgen action via the genitofemoral nerve is not substantiated (13); therefore, it is more likely that testosterone acts directly on the gubernacular growth (22), through the classical AR (cytoplasmic or membrane-associated) or other membrane proteins still to be characterized (18). It would therefore be interesting to explore whether androgen-induced nongenomic pathways play a significant role in the proliferation of the gubernacular cells. If this turns out to be the case, an insight on the mechanisms involved in testis maldescent caused by reduced testosterone levels associated with short CAG tracts will be available.

Additional questions need specific attention, such as the intriguing AR-CAG repeat link between testis maldescent and inguinal hernia. Because both inguinoscrotal descent and inguinal closure are mediated by androgens, common genetic factors may be implicated in both pathological conditions. Given that cryptorchidism is a multifactorial, complex disease with a large spectrum of clinical presentation (13, 23), it is highly likely that distinct genetic anomalies are responsible for different types of testicular maldescent. Consequently, the apparent discrepancies with prior studies are likely to derive from the different inclusion criteria adopted by Davis-Dao et al. (12). Future large case-control association studies focusing on different phenotypic subgroups (with and without inguinal hernia, unilateral and bilateral cases, etc.) will likely answer this question. The study by Davis-Dao et al. (12) indicates a disadvantage only in the case of short CAG repeats; however, upcoming investigations will probably shed light on whether the “optimal range” hypothesis can be applied also to this specific pathological context. In fact, the stratified analysis of nearly 4000 subjects, included in articles dealing with male infertility and AR-CAG length, has provided clinical evidence for the potential benefit of a CAG range corresponding to 22–23 triplets in spermatogenesis (24). However, it must be taken into consideration that this specific range may not be the same across different ethnic groups and may even vary in different tissues because the effect of polyQ repeat on transactivation is cell specific, presumably due to distinct profiles of coregulator proteins (11). Moreover, it is possible that spermatogenesis, more than the process of testis descent, depends predominantly on the genomic action of androgens, and thus on the direct consequence of the CAG length on transactivation. Clearly, more functional studies are needed for the interpretation of clinical data in different types of androgen-dependent diseases.

After a long period of “quiescence” concerning the role of the AR-CAG tract in cryptorchidism, the study by Davis-Dao et al. (12) represents a strong stimulus for further clinical and basic investigations. In addition, this article opens new avenues toward the analysis of other AR-related diseases, which are likely to provide similar unexpected findings.

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References

8. Tut TG, Ghadessy FJ, Trifiro MA, Pinky 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 82:3777–3782
21. Hutson JM, Nation T, Balic A, Southwell BR 2009 The role of the gubernaculum in the descent and undescent of the testis. Ther Adv Urol 1:115–121