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Genetic Variation in the Androgen Receptor Modifies the Association between Testosterone and Vitality in Middle-Aged Men

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Abstract

Background.—Low vitality is a common symptom of testosterone deficiency; however, clinical trial results remain inconclusive regarding the responsiveness of this symptom to hormone replacement.

Aim.—The aim of the present study was to determine if the relationship between circulating testosterone levels and vitality would be moderated by the CAG repeat length in the Androgen Receptor (*AR*) gene, which influences the receptor's sensitivity to testosterone.

Methods.—We examined 676 men in the Vietnam Era Twin Study of Aging (VETSA) when they were, on average, 55.4 years old (SD=2.5). Salivary testosterone levels were measured by three samples collected at waking on three non-consecutive days. The average testosterone level was classified as low, normal, or high based on 1 SD cut-offs. Analyses were conducted using multilevel, mixed linear models, which accounted for the non-independence of the twin data, and adjusted for the effects of age, ethnicity, BMI, chronic health conditions, depressive symptoms, and sleep quality.

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Outcomes.—Vitality was measured using the SF-36 Vitality subscale.

Results.—We observed a significant interaction between salivary testosterone and AR-CAG repeat length. When the repeat length was short, men with low testosterone had significantly lower vitality. As the AR-CAG repeat length increased, the magnitude of the testosterone effect decreased.

Clinical Translation.—The observed interaction between testosterone and variation in the *AR* gene suggests that men with more sensitive androgen receptors, as indicated by a shorter AR-CAG repeat, are more likely to experience symptoms of age-related testosterone deficiency.

Strengths & Limitations.—Strengths of the present study include our use of a large community-based sample, the use of multiple testosterone measurements, and the availability of a comprehensive set of covariates which may impact the association of interest. Limitations include the homogeneous nature of the sample with respect to ethnicity, the brevity of the SF-36 Vitality subscale, and our inability to establish change in testosterone levels due to the cross-sectional nature of data.

Conclusions.—The association between testosterone and vitality appears to be clinically meaningful, and is in part dependent upon variation in the *AR* gene.

Keywords

Low Testosterone; Androgen Receptor Gene; Vitality; Age-related Hypogonadism

1. Introduction

In men, circulating levels of the primary androgen testosterone can begin to decline as early as the mid-thirties [1–4]. In some cases, this age-dependent decline in testosterone biosynthesis can lead to the development of symptomatic hypogonadism [5–7]. The possible symptom presentation of testosterone deficiency is extensive, ranging from decreased libido to reductions in muscle mass and strength [8]. One of the most common symptoms is the subjective complaint of decreased energy or loss of vitality [9]. Indeed, among urologic patients, decreased energy has been shown to be the most commonly reported symptom associated with low testosterone, and was furthermore perceived to be the symptom most likely to benefit from medical intervention [10].

Current clinical practice guidelines recommend the treatment of testosterone deficiency with testosterone replacement therapy (TRT) in order to restore normal testosterone levels as well as improve overall health and quality of life [8, 11]. Despite these guidelines, it remains unclear whether TRT is indeed an efficacious treatment for many of the physiological changes associated with testosterone deficiency in aging men, in particular with regard to energy level or vitality. In a non-randomized study of over 1,400 hypogonadal men receiving TRT, subjective ratings of vitality improved substantially over the course of treatment [12]. Smaller studies of similar design have also shown that TRT is associated with improvement in general health-related quality of life ratings [13, 14]. While these latter studies did not conduct direct assessments of changes in energy levels, both utilized the Aging Males'

Symptoms (AMS) scale, which includes items specific to vitality, as well as mood, sexual functioning, and other symptoms of testosterone deficiency [15].

In contrast to these positive findings, results from the Testosterone Trial, the largest randomized controlled study of TRT conducted to date, provide at best mixed results for the impact of testosterone on subjective energy levels [16, 17]. Following a 1-year course of TRT, investigators did not observe a clinically meaningful difference (defined as a change of 4 or more points) on the FACIT-Fatigue scale [16, 18]. However, a statistically significant difference between the treatment and placebo groups was observed on a secondary measure, the Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36) Vitality subscale [19]. A subsequent meta-analysis of placebo-controlled trials of TRT, which included results from the Testosterone Trial, suggested no impact of testosterone replacement on vitality, whereas significant effects were reported for several aspects of sexual functioning [20].

One factor that might account for the inconsistency of the literature is that treatment effects are being partially obscured due to an interaction between variation in the androgen receptor (AR) and testosterone level. Testosterone primarily influences peripheral tissues and the central nervous system by binding to and activating the AR [21, 22]; however, the interplay of testosterone with the AR is known to differ as a function of a trinucleotide (CAG) repeat located on exon 1 of the AR gene [23, 24]. Normally distributed, and varying in length from 7 to 36 repeats, this polymorphism of the AR gene is strongly associated with the transactivational properties of the AR – the shorter the CAG repeat length, the greater the transactivational effect – meaning that the ligand-dependent effect of the AR on the transcription of androgen responsive genes differs as a function of the repeat length [25, 26]. This effect provides a mechanisms by which the AR-CAG repeat length can be considered a genetic marker of testosterone sensitivity. When utilized as a continuous measure, AR-CAG repeat length has been positively associated with total and free testosterone levels, change in testosterone over time, body fat mass, bone density, insulin and leptin levels, HDL cholesterol, as well as depressive symptoms and measures of negative affect [26–33]. Negative associations with the continuous polymorphism have also been reported for measures of muscle mass, and male infertility [26, 31, 34]. When the polymorphism has been used as a categorical measure (i.e., the AR-CAG repeat is reclassified into short, medium, and long variants), men with the longer variant have been found to have a higher risk of generalized androgen deficiency symptoms compared to men with the shorter variant [35]. Men with shorter variants of the AR-CAG repeat have also been found to be at higher risk for prostate cancer relative to men with longer variants [36, 37].

Testosterone by AR-CAG repeat interactions have been reported for a number of outcomes; however, the directions of the observed interactions, and indeed the interpretations of the interaction effects, have varied depending upon the outcome measure examined. For example, the association between testosterone and depressive symptoms has been found to differ as a function of the AR-CAG repeat such that in men with a shorter repeat length, testosterone levels were inversely associated with depressive symptoms, while no association was observed in men with medium or long repeat lengths [38, 39]. In a sample of adolescent boys (average age 14), testosterone levels were positively associated with levels of risk taking, dominance, and depression in individuals with a short AR-CAG length, while

associations were negative in individuals with a long repeat length [40]. The relationship between testosterone and fat-free body mass has also been shown to differ according to AR-CAG length; however, in this instance the association became stronger as the repeat length increased [41]. Similarly, men with low testosterone and a long AR-CAG repeat length (defined by the authors as greater than or equal to 22) were found to have a greater incidence of metabolic syndrome compared to men with low testosterone and a short repeat length (less than 22) [42]. In the case of insulin sensitivity, higher testosterone was associated with better insulin sensitivity when the AR-CAG repeat was longer (greater than 23), while in individuals with a shorter AR-CAG repeat (less than 23) higher testosterone was associated with poorer insulin sensitivity [43]. No significant association was observed for individuals at the median repeat length of 23.

The present study examined the regulatory effect of the AR-CAG repeat polymorphism on the relationship between testosterone and vitality in middle-aged men. While the consensus view is that fewer CAG repeats in the AR gene result in a more active receptor, the available literature to date provides no clear indication of how this polymorphism in combination with testosterone level is associated with androgen-sensitive traits. We hypothesized that a significant interaction would be observed between testosterone and the AR-CAG repeat length in their association with vitality, such that men with a longer variant of the AR-CAG repeat (i.e. possessing a more insensitive androgen receptor) will be more prone to reduced vitality in the context of low testosterone.

2. Materials and Methods

2.1. Participants

Data were collected during wave 1 of the Vietnam Era Twin Study of Aging (VETSA), a longitudinal study of cognitive and brain aging with baseline in midlife [44, 45]. VETSA participants were randomly recruited from 3322 twin pairs in the nationally representative Vietnam Era Twin Registry, a nationally distributed sample of male twin pairs, both of whom served in the United States military at some point between 1965 and 1975 [46]. Eligibility for the VETSA was conditional on both members of a twin pair agreeing to participate, and being between the ages of 51 and 59 years at the time of recruitment. In total, 1237 men participated in wave 1 of the VETSA. Beginning in year three of the VETSA, funding was obtained to collect neuroendocrine data on the remaining participants (N=795) [45]. Of these, 676 individuals had data on all other relevant variables and were included in the present analyses. Participants were community-based, predominantly Caucasian (89.7%), had an average age of 55.4 years (SD = 2.5, range = 51 to 60), and an average education of 13.8 years (SD = 2.1). While all VETSA participants served in the military, the majority (approximately 80%) did not experience combat situations during their military careers. According to U.S. census data, VETSA participants are similar in lifestyle and health characteristics to American men in their age range[47].

Participants traveled to either the University of California, San Diego or Boston University for a daylong series of physical, psychological, and neuropsychological assessments. On rare occasions (less than 3% of subjects) project staff traveled to the participants in order to complete the assessments. Approval from local institutional review boards was obtained at

all participating sites prior to data collection, and all participants provided signed informed consent upon arriving at the testing site.

Testosterone Collection and Assay—Methods for saliva collection and testosterone immunoassay have been described in detail elsewhere[45, 48]. Briefly, levels of salivary testosterone were obtained via saliva specimens collected on two non-consecutive days at home during a participant's typical week, as well as on an in-lab assessment day. At-home specimens were collected roughly two weeks before the assessment day. Specimens were collected at waking, 30 minutes after waking, 10:00 a.m., 3:00 p.m., and bedtime on all days in order to capture diurnal changes in the hormones of interest (i.e., cortisol, testosterone, and DHEAS). Times of specimen collections were recorded by the participant, and were later confirmed against data from electronic track caps. Once collected, specimens were sent via overnight mail to the University of California, Davis for assay.

Saliva specimens were centrifuged prior to assay at 3000 rpm for 20 minutes to separate the aqueous component from mucins and other suspended particles. Concentrations of free testosterone were determined in duplicate using commercial radioimmunoassay kits (Beckman Coulter Inc., formerly Diagnostics Systems Laboratories, Webster, TX). The least detectable concentrations for the assays were 1.3697 pg/ml (intra-assay coefficient of variation = 3.141, inter-assay coefficients of variation = 4.878). Data from one to three individuals were included in each assay batch, and assays were always performed without knowledge of twin pair zygosity.

Testosterone levels that were greater than 3 SDs above the mean waking measurement were set to missing in order to remove outliers. Data from participants who reported a lifetime history of testicular cancer or a non-skin cancer diagnosis (e.g., leukemia, non-Hodgkin's lymphoma) within one year of their assessment were excluded, as were data from participants who reported taking testosterone supplements or other medications known to alter testosterone levels. Missing data were imputed if a participant had no more than one missing value on a day. To impute missing data, the full samples' mean change in each hormone level between the time-point with the missing value and the adjacent time-point was calculated. The mean change in testosterone for those two points was then added to or subtracted from the participant's non-missing time-point [48]. Imputations were performed for less than 1% of all available hormone measurements. Cronbach's Alphas for the five measurement times across the three assessment days ranged from 0.69 for the bedtime measurement to 0.81 for the waking measurement. Coefficients of variation, an indicator of intra-individual variability, ranged from 21.82 at waking to 28.58 at bedtime. Given its superior reliability and lowest intra-individual variability across the assessment days, we utilized the average waking testosterone measurement for all analyses. Measures of reliability and intra-individual variability for each assessment time are presented in Supplemental Table 1. Once data cleaning was complete, testosterone levels were available for 765 participants. We created a categorical testosterone measure in order to allow us to compare vitality levels in men with low testosterone relative to men with normal or high testosterone. Low testosterone was defined as being at least 1 SD below the mean for the average waking value for all participants (89.1 pg/ml). Based on this definition, 15.2% of the sample was defined as having low testosterone. High testosterone was defined as being at

least 1 SD above the mean for the average waking value for all participants (186.3 pg/ml), and resulted in 13.4% of the participants being placed in this category. All other participants were classified as having normal testosterone.

2.2. Androgen Receptor Genotype

The trinucleotide (CAG) repeat polymorphism in the AR gene was determined using an ABI PRISM 3100 genetic analyzer (Applied Biosystems, Foster City, CA, USA) with fluorescently labeled oligonucleotides and an internal standard (ROX 500) following the method protocol described by Akinloye and colleagues[49]. DNA were obtained from whole blood that was collected at the time of the in-lab assessment and stored for future use. To determine the number of repeats, a 25 μl polymerase chain reaction (PCR) reaction was performed with 12.7 μl of water, 2.5 μl of buffer (1.5 mM MgCl2), 5 μl Q-solution, 0.3 μl of Gold Taq (5 U/μl), 1 μl of deoxynucleotide mixture (10 mM), 1 μl each of both forward and reverse primer and 1.5 μl genomic DNA subjected to 30 cycles of PCR amplification. The CAG primers were 5'-TCC AGA ATC TGT TCC AGA GCG TGC-3' and 5'-GCT GTG AAG GTT GCT GTT CCT CAT-3'. PCR amplification was performed under the following conditions: 94 C for 45 sec, 59 C for 30 sec and 72 C for 1 minute using the ABI Veriti Thermocycler. PCR products were separated by capillary electrophoresis in a POP-4 (4% poly-dimethylacrylamide, 8 M urea, 5% 2-pyrrolidinone) gel. Allele assignment was done on an ABI 3100 genetic analyzer with ABI Genotype software after addition of the internal standard Genescan ROX 500 to the probe (all from Applied Biosystem, Foster City, CA, USA). Each run incorporated probes from previously sequenced DNA as positive controls. The output data was analyzed with GeneScan Analysis software and Genotyper software (Applied Biosystems) to determine the number of CAG repeats. Of the 765 VETSA participants with analyzable testosterone data, DNA for AR genotyping was available for 717 individuals. Consistent with the literature on the AR CAG repeat [24, 26], the median repeat length for the sample was 22, and the range was 8–37 (see Figure 1).

2.3. Assessment of Vitality

Participants completed a questionnaire packet prior to the in-lab assessment day that included the SF-36 [19]. The SF-36 Vitality subscale reflects participants' levels of energy and fatigue over the past four weeks on four Likert-scale items. Low scores indicate that the individual "feels tired and worn out", whereas high scores indicate that the individual feels "full of pep and energy". The scale has been shown to be sensitive to conditions such as depression, chronic obstructive pulmonary disease, and anemia, with 5–10 point differences between groups representing clinically meaningful effects [50]. Due to negative skewness of the measure, raw scores were subtracted from the maximum value plus one, and were then square-root transformed. The resulting value was multiplied by negative one in order to maintain a positive correlation with the original raw score.

2.4. Covariates

All analyses adjusted for the potential confounding effects of age, race/ethnicity, body mass index (BMI), chronic health conditions, overall sleep quality, and depressive symptoms. Covariates were selected based on established associations with testosterone level, AR-CAG repeat length, as well as potential direct or indirect effects on vitality. Because African

Americans have been shown to have a slightly shorter AR-CAG repeat length [24, 26], participants were classified as African American or Non-African American. This latter group included participants who identified as white non-Hispanic, as well as Hispanic or Latino. BMI was calculated as weight (kg) divided by height squared (m^2) based on objective measurements obtained on the in-lab assessment day. Chronic health conditions were assessed through an in-person medical history interview. Each participant was asked whether a physician had ever told him he had any of 49 medical conditions/illnesses. A composite score reflecting 16 chronic major health problems known to negatively influence mortality was then created based on items from the Charlson Comorbidity Index [51]. Sleep quality was assessed with the Pittsburgh Sleep Quality Index (PSQI), a well-validated measure of sleep quality and disturbance over the preceding month [52]. The PSQI consists of 19 items assessing sleep quality, latency, duration, efficiency, disturbance, use of sleep medications, and daytime dysfunction. We utilized the global score from the PSQI as an overall measure of sleep quality. Depressive symptoms were assessed with the Center for Epidemiological Studies Depression Scale (CES-D) [53]. The CES-D consists of 20 items relating to the frequency of specific moods and behaviors over the past week. Because the CES-D contains items addressing the somatic manifestations of depression, which would be expected to correlate strongly with vitality, we utilized the 7 mood-specific elements of the instrument and excluded other items. Scores range from 0 to 21, with higher scores indicating more severe depressive symptoms.

2.5. Statistical Analyses

Analyses were conducted using multilevel, mixed linear models in SAS (SAS Proc Mixed, SAS version 9.3), which allowed for the use of data from all available participants while correcting for the non-independence of the observations (i.e., twin clustering). Each hormone assay batch was assigned a unique identification number (referred to as batch ID) so that we could further control for any potential clustering introduced by the laboratory procedures. Both family (twin clustering) and batch were entered into the model as random effects. Significant main and interaction effects were determined using type III test of fixed effects, indicating the unique association of each element of the model independent of the others. Significance level was set at alpha = 0.05 for all main and interaction effects. In order to assist with the interpretation of results, the transformed vitality score was standardized to a mean of 0 and a variance of 1.0 prior to analysis.

3. Results

Characteristics of the VETSA subsample with testosterone data, as well as differences among the low, normal and high testosterone groups are presented in Table 1. After accounting for missing data among our covariates, data were available for 676 participants in total, 103 with low testosterone, 484 with normal testosterone, and 89 who were classified as having high testosterone. The average free testosterone levels for the groups were 74.1 pg/ml (SD=13.4), 134.2 pg/ml (SD=25.3), and 227.8 pg/ml (SD=35.1), respectively.

There were significant main effects of testosterone on AR-CAG repeat length, sleep and vitality. Participants with low testosterone had poorer overall sleep quality (p = .03), and

reported lower vitality scores (p = .03). The high testosterone group had longer AR-CAG repeat length relative to the low and normal testosterone groups (p < .01). The AR-CAG repeat length was significantly, positively, associated with testosterone level (p < .01), but was not related to any of the selected covariates or vitality. All of the covariates had a significant association with vitality.

Results from the mixed effects models, both with and without the interaction effect, are presented in Table 2. After controlling for all covariates we observed a significant interaction effect between testosterone and AR-CAG repeat length (p = .03). The main effects of testosterone and the AR-CAG repeat were themselves not significant. The nature of the interaction is such that when the CAG repeat length is short, testosterone has a significant negative association with the Vitality subscale. As the CAG repeat length increases, the association between testosterone and Vitality is lost. A representation of the interaction is presented in Figure 2. For purposes of illustration, we created three categories representing participants with 20 or fewer CAG repeats (short), 21 and 23 repeats (middle), and 24 or more repeats (long). Raw (untransformed) Vitality subscale scores are presented in order to ease interpretation of the results. In the low testosterone group, AR CAG repeat length was significantly associated with vitality. Participants with low testosterone and short repeat lengths (20 or fewer) had significantly lower vitality scores relative to participants with low testosterone and long repeat lengths (24 or more, p =.015). In both the middle or long repeat length groups, we observed no effect of testosterone on vitality (p = .868; p=.325, respectively).

4. Discussion

Decreased energy or loss of vitality is a common symptom of age- or disease-related testosterone deficiency [9]. Despite the prominence of this view, neither the role of testosterone in governing the magnitude of diminished vitality in community-based samples of older men, nor the role of genetically determined androgen receptor sensitivity in the efficacy of TRT for energy-related complaints has been carefully investigated [12–14, 16]. In the present study we found a significant interaction effect between CAG repeat length in the AR gene and salivary testosterone levels with regard to self-reported vitality. Counter to our hypothesis, we found that at middle or high levels of testosterone, differences in the AR gene CAG repeat polymorphism were not associated with vitality, but when testosterone was low, having a longer CAG repeat length was significantly associated with greater vitality. Thus, an adequate level of testosterone may be enough to compensate for differences that may be due to AR CAG repeat length. However, individuals who may have some testosterone deficiency appear to be more strongly affected by differences in AR CAG repeat length. This interaction effect was observed after accounting for the potential confounding effects of chronic health conditions, BMI, sleep quality, and depressive mood symptoms, all of which may impact ratings of vitality and have been associated with testosterone deficiency. It should be noted that in what may be considered the more standard analysis, one that only included main effects but no interactions, neither testosterone nor AR-CAG repeat length was associated with vitality. Thus, our results show that the relationship between testosterone and vitality is obscured if the interaction between testosterone and the

AR polymorphism is not considered. More specifically, it obscures that relationship in individuals with low testosterone.

The present finding may initially appear counterintuitive, indeed it is inconsistent with our initial hypothesis; however, the nature of our observed interaction effect is nevertheless consistent with previous reports of testosterone by AR-CAG repeat interactions in relation to overall depressive symptoms in men [38, 39]. In those studies, men with low testosterone levels and shorter AR-CAG repeat lengths had greater likelihoods of clinically meaningful depressive symptoms, as measured by the CES-D, in comparison to men with similar testosterone levels and longer AR-CAG variants. Somatic complaints that are consistent with loss of energy or decreased vitality are a common feature of depression. As previously noted, the CES-D employs several items that target the somatic aspects of depression, items that were removed from the scale for the present analyses. It is possible that the similarity in the interaction effects observed in prior studies of depression and the present study reflect this commonality between measures of depression and vitality. The direction of the interaction effect for vitality and for depression is also consistent with studies of TRT for metabolic markers, sexual functioning, and prostrate growth (see below).

Studies that have examined the interaction between testosterone and the AR-CAG repeat in relation to measures of body composition (fat free mass and fat mass) and metabolic functioning (insulin sensitivity and metabolic syndrome) have found effects that go in the opposite direction of the present result [29, 41, 43]. In other words, lower testosterone levels were associated with poorer health outcomes when the AR-CAG repeat was longer rather than shorter. The authors argued that a more active AR (i.e., one with a shorter CAG repeat length) will be more able to utilize available testosterone, resulting in fewer functional changes when the hormone level becomes reduced [54]. Our finding and the aforementioned depression study run counter to this hypothesis, and suggest that, at least with regard to vitality or depression, men with both low testosterone and putatively greater androgen sensitivity are be more likely to show the symptoms of age- or disease-related testosterone deficiency.

The conflicting results represent a challenge in terms of understanding how the interaction between testosterone and the AR-CAG repeat may impact health and disease. To date, the interaction has been examined in so few studies that it is difficult to discern a clear pattern of effects and whether there are similarities among the domains that show an effect in one direction versus another. Additional studies are clearly warranted in order to increase the number of data points in this regard. It has also been established that androgens and AR signaling have tissue- and cell-specific actions, and that after binding with its ligand the AR can either activate or repress androgen responsive genes [55–57]. The linear effect of the AR-CAG repeat on transcriptional activity of the AR has also been called into question, with some evidence suggesting that median rather than short repeat length may result in optimal receptor activity [58]. Finally, a factor that is important to keep in mind is that the conceptual model for the interaction between testosterone and variation with the AR gene (represented here and in other studies as the AR-CAG repeat length) is biologically incomplete, as it does not include the possible contributions of AR co-regulators. In addition to the AR-CAG repeat length, the AR's activity is influenced by a class of proteins known as

nuclear receptor co-regulators [59–61]. These proteins function to either increase or decrease the transactivational properties of the receptor, similar to effects of the AR-CAG repeat length[62]. A potential explanation for the disparate results may be that the so far unaccounted for AR co-regulators are differentially expressed across functional domains, and as a result may alter the way in which testosterone and the AR interact with each other.

While not a demonstration of TRT efficacy, we believe that the findings from the present study have implications for the use of testosterone replacement as a form of treatment. Within our low testosterone group, substantial differences in vitality levels were observed as a function of the AR-CAG repeat. These differences were statistically significant, and were also clinically meaningful (10 point difference) for the SF-36 Vitality subscale [50]. Based on these differences, we speculate that when it comes to the treatment of energy- or vitality-related complaints, low testosterone individuals with shorter AR-CAG repeat lengths may be the most likely to benefit from TRT. Results from a handful of TRT studies lend support to this speculation. In a small study (N=15) of men with postsurgical hypogonadeotropic hypogonadism (i.e., testosterone deficiency that is secondary to removal of pituitary adenoma), shorter AR-CAG repeat length was associated with improvement on a number of metabolic markers following TRT [63]. In a separate, larger sample (N=73) of men with late-onset hypogonadism, shorter AR-CAG repeat length was associated with improvements in sexual functioning following TRT [64]. Finally, in hypogonadal men undergoing TRT, a shorter AR-CAG repeat length was associated with greater rate of prostate growth and prostate size [65]. It remains to be seen whether the evaluation of TRT effects based on AR-CAG repeat length would generate similar patterns of results for all outcomes of interest. As previously noted, tests of the interaction between testosterone and the AR polymorphism have shown that different associations with the hormone can be observed at long and short repeat lengths, with opposing patterns about equally split among studies [38, 39, 41–43, 66]. While additional research is clearly warranted, our current results as well as the findings from previous studies strongly argue for the inclusion of the AR-CAG repeat as a critical moderating factor in any examination of TRT.

It is worth noting that our definition of low testosterone is based on a statistical cut-off (1 SD or more below the mean for the average waking value) and not an established clinical threshold. Nevertheless, the rate of low testosterone we obtained with this definition (15.2%) is well within the range of reported prevalence rates of age-related or late onset hypogonadism [5, 6]. Despite the abundance of interest in hypogonadism as a prominent and potentially treatable condition of male aging, epidemiological data regarding the condition are lacking. Thus, it remains unclear whether our rates are an under- or over-estimate of low testosterone for men in this age range. It is also the case that we utilized saliva-based measures of testosterone rather than blood-based measurements of total or bioavailable testosterone. Indeed, salivary testosterone has been found to correlate .71 with serum-based measures of free testosterone [67]. While this fact may limit the degree to which our results can be directly compared to those from other studies, we believe that our method of testosterone measurement represents an important strength of the present study. Our measure of testosterone was based on an average of measurements taken on three non-consecutive days, which would make our definition of low testosterone more reliable than one based on a single assessment. The use of free testosterone rather than total testosterone should also be

considered advantageous, as findings from the European Male Aging Study (EMAS) have shown that free testosterone is associated with symptoms of testosterone deficiency, including lower vitality, even among men with otherwise normal total testosterone levels [68].

Results from the present study should be considered in the context of the following limitations. The VETSA is a relatively homogenous sample with respect to ethnicity, and we cannot rule out that differences in the AR-CAG repeat, as well as its impact on the AR itself may differ across different ethnic groups. The SF-36 Vitality subscale is brief, consisting of only 4 Likert-scale items. Nevertheless, this subscale of the SF-36 is well validated and widely used, and we did observe clinically meaningful differences on this measure. Finally, it is also the case that the cross-sectional nature of our data limits our ability to determine whether our observed testosterone levels are indeed the result of age-related changes, or are long standing.

5. Conclusion

Within a community-based sample of middle-aged men, we found evidence for a clinically meaningful association between testosterone and self-reported vitality, but only after accounting for the interaction between testosterone and variation in the AR gene. Contrary to predictions, an association between vitality and testosterone level was observed for men with a shorter AR-CAG repeat length, suggesting that men with a more testosterone-sensitive AR may be more likely to experience symptoms associated with age-related testosterone declines. Accounting for this critical interaction effect may have implications for the efficacy of TRT in men with age-related hypogonadism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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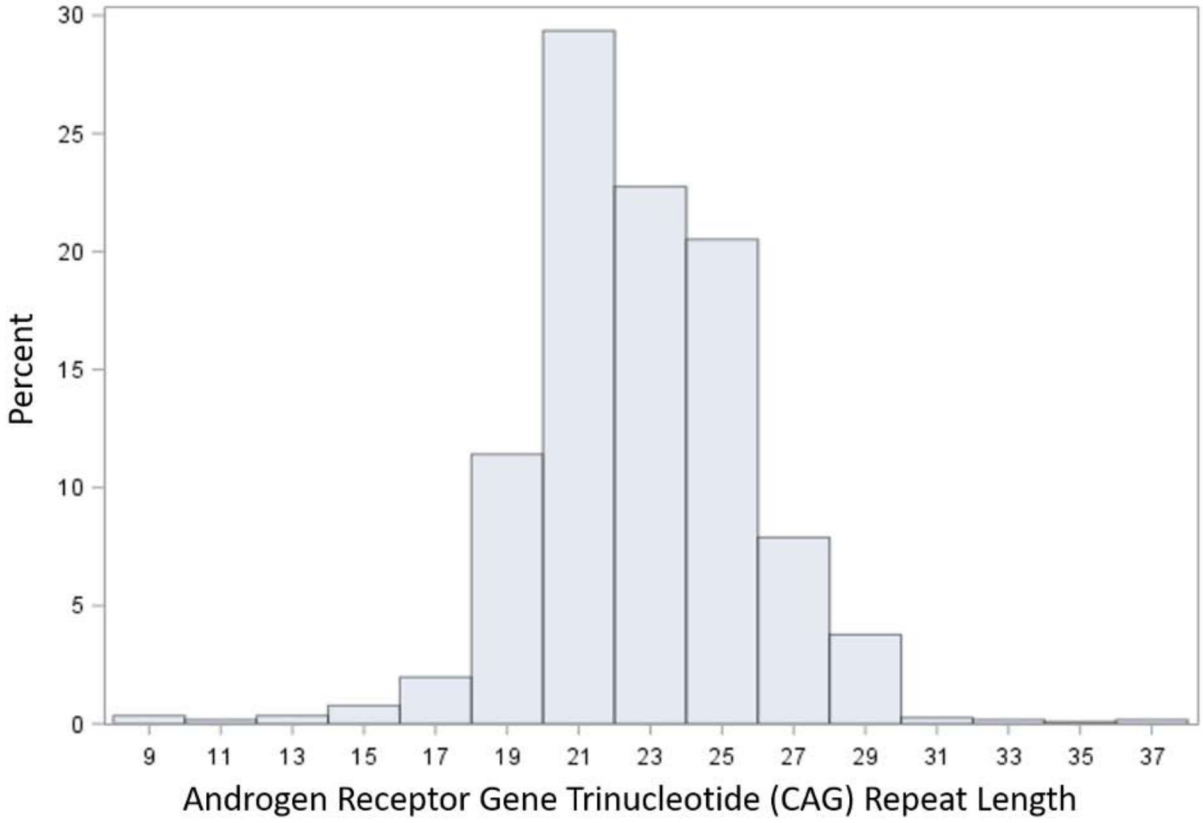

Figure 1.
Distribution of Androgen Receptor Gene CAG repeat length in the Vietnam Era Twin Study of Aging (VETSA) sample.

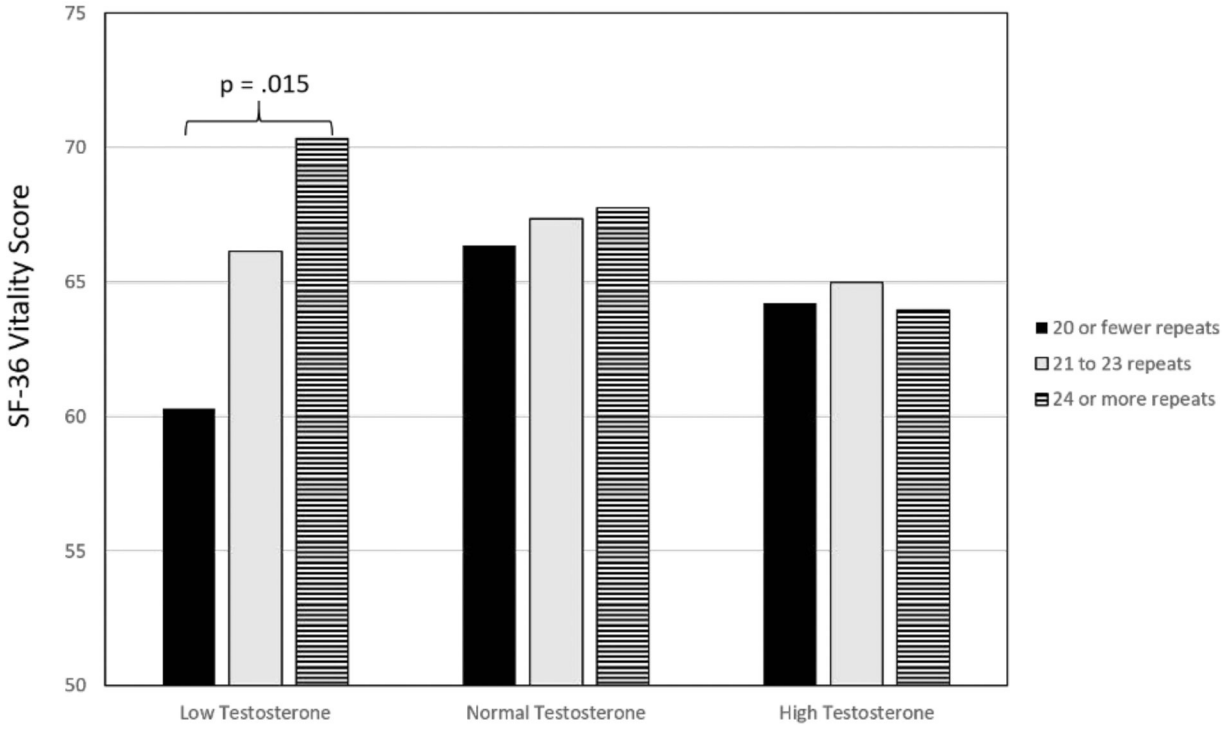

Figure 2.
Association of CAG repeat length with vitality by testosterone group.

Table 1.

Sample characteristics and differences between testosterone groups

	Full Sample (N = 676)	Low Testosterone (N = 103)	Normal Testosterone (N = 484)	High Testosterone (N = 89)	p
Age (years)	56.4 (2.6)	56.8 (2.5)	56.4 (2.6)	56.0 (2.6)	.06
Race (% African American)	4.3%	4.9%	5.0%	0.0%	.13
BMI (kg/m^2)	29.2 (4.8)	30.0 (5.3)	29.2 (4.7)	28.6 (4.7)	.15
Health Conditions	1.0 (1.1)	1.3 (1.2)	1.0 (1.1)	1.1 (1.1)	.13
PSQI Sleep Quality	5.1 (3.5)	5.9 (4.1)	5.0 (3.5)	4.8 (2.8)	.03
Depressive Mood Symptoms	1.8 (3.1)	2.2 (3.4)	1.7 (3.1)	1.8 (2.7)	.31
Average Salivary Testosterone (pg/ml)	137.5 (48.5)	74.1 (13.4)	134.2 (25.3)	227.8 (35.1)	<.01
AR CAG Repeat Length	22.2 (3.3)	22.1 (3.0)	22.0 (3.4)	23.4 (3.0)	<.01
SF-36 Vitality Score	62.1 (19.0)	58.5 (21.6)	63.3 (18.7)	59.91 (16.6)	.03

Reported p values are based on mixed effects models comparing the low, normal, and high testosterone groups, while accounting for the correlated nature of the twin data. For ease of group comparison, vitality scores are presented on their raw scale. Standard deviations are presented in parentheses.

Table 2.

Mixed model results for associations with SF-36 Vitality with and without the testosterone by AR-CAG repeat interaction

	No Interaction Model			Interaction Model		
	F	**DF**	**p**	**F**	**DF**	**p**
Age	0.47	1, 290	.4931	0.27	1, 288	.6055
Race	10.61	1, 290	.0013	10.21	1, 288	.0016
Health Conditions	12.34	1, 290	.0005	12.49	1, 288	.0005
BMI	66.21	1, 290	<.0001	68.13	1, 288	<.0001
PSQT Sleep Quality	11.85	1, 290	.0007	13.88	1, 288	.0002
Depressive Mood Symptoms	33.63	1, 290	<.0001	33.39	1, 288	<.0001
AR-CAG Repeat Length	2.07	1, 290	.1510	3.37	1, 288	.0674
Testosterone	2.05	2, 290	.1312	1.36	2, 288	.2572
Testosterone x AR-CAG Repeat	--	--	--	3.50	2, 288	.0314

F and *p* values indicate the type III test of fixed effects, controlling for all other elements of the model.