

Androgen receptor CAG repeat polymorphism and hypothalamic-pituitary-gonadal function in Filipino young adult males

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Abstract

Objectives: Testosterone (T), the primary androgenic hormone in males, is stimulated through pulsatile secretion of LH and regulated through negative feedback inhibition at the hypothalamus and pituitary. The hypothalamic-pituitary-gonadal (HPG) axis also controls sperm production through the secretion of follicle-stimulating hormone (FSH). Negative feedback in the HPG axis is achieved in part through the binding of T to the androgen receptor (AR), which contains a highly variable trinucleotide repeat polymorphism (AR-CAGn). The number of repeats in the AR-CAGn inversely correlates with transcriptional activity of the AR. Thus, we predicted longer AR-CAGn to be associated with higher T, LH, and FSH levels.

Methods: We examined the relationship between AR-CAGn and total plasma T, LH, and FSH, as well as “bioavailable” morning (AM-T) and evening (PM-T) testosterone in 722 young (21.5 ± 0.5 years) Filipino males.

Results: There was no relationship between AR-CAGn and total T, AM-T, or LH ($P > .25$ for all). We did observe a marginally non-significant ($P = .066$) correlation between AR-CAGn and PM-T in the predicted direction, and a negative correlation between AR-CAGn and FSH ($P = .005$).

Conclusions: Our results both support and differ from previous findings in this area, and study parameters that differ between our study and others, such as participant age, sample time, and the role of other hormones should be considered when interpreting our findings. While our data point to a modest effect of AR-CAGn on HPG regulation at best, the AR-CAGn may still affect somatic traits by regulating androgenic activity at peripheral tissues.

KEYWORDS

androgen receptor, follicle-stimulating hormone, HPG axis regulation, short tandem repeats, testosterone

1 | INTRODUCTION

Testosterone (T), the primary sex steroid produced in men by the testes, exerts effects on the male body, reproductive system, and brain through the binding of the androgen receptor (AR; Chang, 2002). Within the expressed AR protein are two trinucleotide short-tandem repeat (STR) regions that are highly polymorphic in humans, including a polyglutamine

tract (AR-CAGn) located in the N-terminal transactivation domain (Palazzolo et al., 2008). In the healthy range of AR-CAGn in humans (6–39 repeats), longer repeat length is associated with decreased transcriptional activity in response to T (Buchanan et al., 2004; Kazemi-Esfarjani et al., 1995; Simainen et al., 2011). Consistent with this effect, behavioral and phenotypic traits in men with longer AR-CAGn often resemble those seen in cases of hypogonadism or other kinds

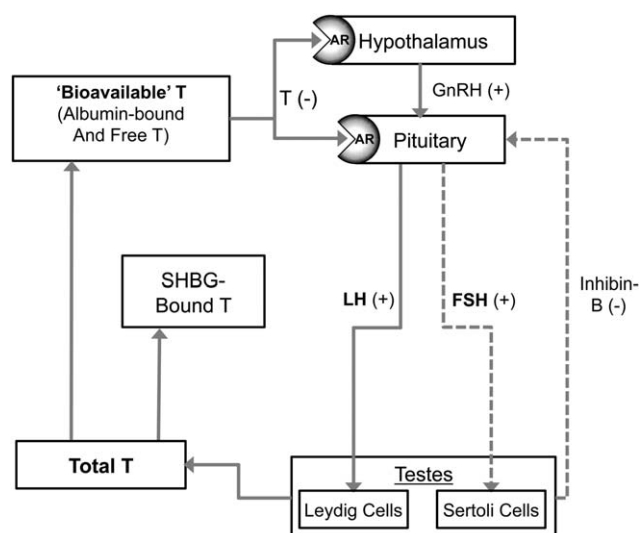


FIGURE 1 Schematic depiction of HPG axis regulation in human males. HPG axis endocrine measures investigated in the current study include LH, FSH, total plasma testosterone (Total T), salivary bioavailable testosterone (Bioavailable T), and variation in the polyglutamine repeat in exon 1 of the androgen receptor (AR; AR-CAGn). Solid gray arrows follow the regulatory portion of the HPG axis directly involving testosterone and the AR, while the negative feedback loop involving inhibin-B is shown using dashed arrows

of androgen insensitivity (Nieschlag, Behre, & Nieschlag, 2004; Walsh et al., 2005; Zitzmann et al., 2001).

In addition to its role on target tissues, AR-CAGn could also modify circulating T levels by altering the feedback sensitivity of the hypothalamic-pituitary gonadal (HPG) axis (Figure 1). The HPG regulates testicular T production through negative feedback, with hypothalamic gonadotrophin releasing hormone (GnRH) triggering pituitary luteinizing hormone (LH) secretion, stimulating T production and secretion in the Leydig cells. Binding of T to the AR in the hypothalamus and pituitary, in turn, attenuates further T production by reducing GnRH and LH output (Norman and Litwack, 1997). Longer AR-CAGn could thus reduce the feedback sensitivity of the HPG axis, which would be expected to increase both T and LH. Circulating levels of follicle stimulating hormone (FSH)—mediated in part through the same T-driven negative feedback inhibition as LH but also through the inhibitory feedback of inhibin B—should as a result be less closely linked to AR-CAGn (Figure 1).

Previous work examining the relationship between AR-CAGn and HPG regulation provide mixed support for an effect of AR-CAGn on the HPG axis. While several studies have reported increased T with AR-CAGn (Crabbe et al., 2007; Huhtaniemi et al., 2009; Lindstrom et al., 2010; Walsh et al., 2005), other studies have failed to detect this relationship (Campbell et al., 2009; Canale et al., 2005; Goutou, Sakka, Stakias, Stefanidis, & Koukoulis, 2009; Skjaerpe,

Giwercman, Giwercman, & Svartberg, 2008; Van Pottelbergh, Lombroso, Goemaere, Sultan, & Kaufman, 2001). The reasons for inconsistencies across studies are unclear, but are compounded by three distinct measures of T: total T, free T, and “bioavailable” T. Of the total testosterone circulating in the plasma (total T), the vast majority is bound to either sex hormone binding globulin (SHBG; 50–60%) or albumin (40–50%); the remaining 1–2% is unbound or “free” T. Relative to albumin, SHBG has a particularly high binding affinity for T (Norman and Litwack, 1997). As a result, only the free and albumin-bound fractions of circulating testosterone are readily bioavailable for biological activity—including mediating negative feedback control of the HPG axis (Nieschlag et al., 2004). Differences in hormone measures do not appear to explain all cross-study inconsistencies, however, and other confounding factors such as participant age or population genetic substructure also appear to be important contributing factors (Crabbe et al., 2007).

Here we use genetic and hormonal data from a large sample ($n = 722$) of young adult males (20.5–22.5 years old) enrolled in the Cebu Longitudinal Health and Nutrition Survey to assess the role of AR-CAGn in the regulation of HPG axis function. In addition to total T, LH, and FSH measured in plasma, we also measured salivary T from samples obtained immediately after waking and before bed—each corresponding to the highest and lowest points in testosterone circadian fluctuations, respectively. Salivary samples also capture bioavailable T, which includes both free and albumin-bound testosterone, but not T bound by high-affinity SHBG. We predicted that longer AR-CAGn would lead to reduced HPG feedback sensitivity, thus elevating total T, bioavailable T, and LH.

2 | METHODS

Data come from participants in the Cebu Longitudinal Health and Nutritional Survey (CLHNS), located in Cebu City, Philippines (Adair et al., 2011). The present analyses focus on the 722 men for whom all necessary 2005 questionnaire data and biomarkers of HPG function and AR-CAGn polymorphism data were available and met selection criteria described below. This research was conducted under conditions of written informed consent with human subjects clearance from the Institutional Review Boards of Northwestern University and the University of Chapel Hill, North Carolina.

2.1 | Control variables

Following our past work in this cohort (Kuzawa, Gettler, Muller, McDade, & Feranil, 2009), men were classified as pair-bonded if they were currently living with a partner and/or were legally married. Fathers were defined as men who were living with one or more son or daughter. Because circadian hormone profiles are altered by abnormal sleep-wake

cycles, we excluded men with abnormal sleep cycles using criteria described previously (Desantis et al., 2015).

2.2 | Plasma total testosterone, luteinizing hormone (LH), and follicle stimulating hormone (FSH)

Participants were asked to fast overnight for 12 hours, and blood samples were collected in clinics the following morning using EDTA-coated tubes. Mean time of blood draw was 07:07 (range 05:40–09:30). After separation, samples were frozen and shipped on dry ice to Northwestern University for analysis. Plasma total testosterone was analyzed with a commercially available enzyme immunoassay (Diagnostic Systems Laboratories #DSL-10-4000, Webster, TX), as were plasma LH (Immuno-Biological Laboratories #IB19104, Minneapolis, MN) and plasma FSH (40-056-205005; Genway Biotech). All samples were assayed in duplicate, and control samples were included with each assay to monitor between-assay variation. The coefficients of variation for low and high controls were, respectively, 13.3% and 5.8% for total T, 5.7% and 8.0% for LH, and 7.2% and 12.6% for FSH.

2.3 | Salivary testosterone measurement

Saliva and plasma samples were taken during the same 24-hour period. Each participant was provided with instructions and two tubes for saliva collection. The first sample was collected immediately prior to bed (PM-T). After collection, tubes were sealed and kept at room temperature. Participants were instructed to place the second tube next to their bed and to collect the second sample immediately upon waking the following morning (AM-T). At each collection time, the participant was asked to record the time of collection, with average PM-T and AM-T collection times being 22:23 and 06:34, respectively. Tubes were collected later that day, and immediately placed on ice packs in a cooler by an interviewer. Tubes were then transported to a freezer where they were stored at -35°C until shipment on dry ice to the Laboratory for Human Biology at Northwestern University, where they were stored at -80°C . Samples were thawed, centrifuged, supernatant separated, and aliquoted into smaller tubes for subsequent analysis of individual analytes. Salivary T concentrations were determined in duplicate using an enzyme immunoassay protocol developed and validated for use with saliva samples (Salimetrics #1-2402, State College, PA). The between-assay coefficients of variation were 5.6% and 6.7% for high and low controls, respectively.

2.4 | AR-CAGn measurement and quality control

DNA samples were run on 384-well microtiter plates. For quality control, we randomly placed three Centre d'Etude du Polymorphisme Humain (CEPH) control DNA samples, isolated from lymphoblast cell lines, in each quadrant of the 384-

well plates. The CAGn repeat in the androgen receptor (AR) gene was amplified using a previously validated protocol (Ackerman et al., 2012) with a fluorescently labeled primer (AR CAGn forward, 5'-NED-GTGC GCGAAGT GATCCAGAA-3'; and reverse, 5'-TAGCCTGTGGGG CCTCTACG-3'). For each PCR, 20 ng of genomic DNA was amplified in a total volume of 9.2 μL in the presence of 200 μM deoxynucleotide triphosphate, 1.5 mM MgCl_2 , 0.7 U AmpliTaq Gold polymerase, and 0.75 μM of forward primer and 0.75 μM of reverse primer. PCR products were electrophoresed in the presence of an internal size standard (GeneScan 500 ROX) at room temperature on the Applied Biosystems 3130XL Capillary DNA sequence analysis system, and genotypes were assigned using the GENEMAPPER software version 4.0 (Applied Biosystems). We also visually examined the chromatogram for each sample to confirm the genotype assignment. The distribution of AR-CAGn in this sample was similar to that of a separate East Asian population run in the same laboratory using these protocols (Ackerman et al., 2012), but shifted slightly towards shorter repeat length.

Several measures of quality control were conducted on the larger database ($n = 1106$) of which this data is a subset. This larger study measured AR-CAGn in both males and females, including in 177 mother-offspring dyads. In the original dataset, 18 samples had ambiguous genotyping calls which were not clearly consistent with three base pair repeats (e.g., an allele which was in between 31 and 32 repeats in size) and were excluded. Forty-one samples had quality control replicates which the individual running laboratory analyses and determining genotyping calls was blinded to. All of these 41 sample quality control replicate pairs matched. Four of the 854 male individuals showed a second AR-CAGn allele and were dropped since males are only expected to show one allele on the AR-CAGn locus, an X-linked gene. These heterozygous males likely represent sample contamination or mix-up, but we note that CNV gains have been observed at the AR locus in healthy individuals (Database of Genomic Variants, <http://dgv.tcag.ca/>, accessed on July 11, 2014). Ninety-nine percent (176 of 177) of mother-offspring pairs contained at least one matching allele. The single mother-offspring pair without the expected matching allele was dropped from the analysis.

To evaluate whether population structure might be a determinant (and potential confounder) of AR-CAGn allele frequencies, principal components (PCs) of genome-wide genetic variation were considered. The derivation of these principal components have been described previously (Wu et al., 2012). The bivariate association between the first ten PCs and AR-CAGn were examined. In these analyses each female individual was considered twice, once for each of the two alleles. Neither among mothers nor the offspring were any of the first 10 PCs significantly ($\alpha = 0.05$) associated with AR-CAGn allele frequency. Given this lack of apparent

confounding population substructure, PCs were not included in subsequent analyses.

2.5 | Statistical analysis

Prior to analysis, the data for males only were examined for outliers and influential observations (Zuur, Ieno, & Elphick, 2010). One individual with AR-CAGn outside the normal range (6–39) (Buchanan et al., 2004) was excluded, as were individuals with AM-T samples >600 pg/mL (suggesting oral blood contamination), PM-T levels <2.65 pg/mL, FSH levels <0.002 ng/mL, and LH levels <0.15 mIU/mL, each falling below normal physiological or assay detection limits. From our original dataset of 734 males, we were left with a sample size of 722 for all analyses. Post-test diagnostics indicated that models using untransformed hormone data were widely associated with heteroscedastic and non-normal residual distributions (Fox, 2002). Model assumptions were met by using natural-log transformed values for plasma T, salivary AM-T, salivary PM-T, LH, and FSH, and so all were log-transformed. We calculated descriptive statistics on unlogged data before using OLS linear regression models to evaluate relationships between androgen receptor CAG repeat length and each measure of HPG function, adjusting for the time of sample collection. All reported results are for log-transformed hormones, while AR-CAGn was untransformed and treated as a continuous variable. Because our prior work has shown that fathers and pair-bonded men may have different T levels (Kuzawa et al., 2009), we considered current fatherhood and pair-bonding status, as well as age, as control variables. Pair-bonded status and age had no qualitative effect on any models and so were excluded from the final analysis, though time of sample and fatherhood status were retained in all. Statistical analyses, diagnostics, and graphics were conducted using R (R Core Development Team, 2011).

3 | RESULTS

3.1 | Hormone Associations

Plasma LH and FSH levels were positively correlated ($F_{3,718} = 69.47$, $P < .001$, $\eta_p^2 = 0.090$), as predicted by their mutual regulation through hypothalamic GnRH. The stimulatory effect of LH on T, and the partially independent regulation of LH and FSH through T and inhibin B, respectively, were detected in the positive correlation between plasma T and LH ($F_{4,717} = 18.78$, $P < .001$, $\eta_p^2 = 0.026$) and the negative correlation between plasma T and FSH ($F_{4,717} = 7.34$, $P = .007$, $\eta_p^2 = 0.010$). Because salivary measures of bioavailable AM-T and PM-T were correlated with total (i.e., bound and free) plasma T levels ($F_{4,717} = 7.37$, $P = .007$, $\eta_p^2 = 0.010$ and $F_{4,717} = 18.78$, $P < .001$, $\eta_p^2 = 0.026$, respectively), we also examined the relationship between salivary T and plasma LH/FSH. PM-T showed the similar positive

TABLE 1 Summary characteristics of study sample examining HPG axis regulation in young Filipino males ($N = 722$)

	Mean (SD)	Range
Age (years)	21.5 (0.3)	20.8–22.6
Father (%)	20.5%	-
Married (%)	25.9%	-
AR-CAGn	21.1 (3.5)	8–38
Salivary AM-T (pg/mL)	194.25 (77.13)	26.12–588.43
Salivary PM-T (pg/mL)	117.00 (53.31)	15.53–406.49
Plasma T (ng/mL)	8.13 (4.05)	3.41–65.43
Plasma LH (mIU/mL)	10.28 (4.87)	0.82–48.37
Plasma FSH (mIU/mL)	3.04 (2.41)	0.89–30.20

and negative correlations with LH ($F_{4,717} = 3.57$, $P = .059$, $\eta_p^2 = 0.005$) and FSH ($F_{4,717} = 9.53$, $P = .002$, $\eta_p^2 = 0.013$) as plasma T. Perhaps because of greater variation arising from the testosterone awakening response (Table 1), salivary AM-T was not related to either LH ($F_{4,717} = 0.98$, $P = .320$) or FSH ($F_{4,717} = 1.09$, $P = .298$), although the direction of the trend was the same as for plasma T and PM-T.

3.2 | Hormone - AR-CAGn Associations

Because AR-CAGn modulates the transcriptional activity of the androgen receptor in response to T—possibly attenuating the negative feedback regulation of the HPG axis—we predicted that longer AR-CAGn would be associated with higher LH, FSH, and T levels. We found mixed support for this hypothesis (Table 2). AR-CAGn was not associated with LH ($F_{3,718} = 0.028$, $P = .867$; Figure 2A) or plasma T ($F_{3,718} = 0.38$, $P = .538$; Figure 2B). However, AR-CAGn was marginally (but non-significantly) positively correlated with salivary PM-T ($F_{3,718} = 3.39$, $P = .066$; Figure 2C), but not with AM-T ($F_{3,718} = 1.14$, $P = .284$; not shown). AR-CAGn was significantly and negatively correlated with FSH ($F_{3,718} = 8.07$, $P = .005$, $\eta_p^2 = 0.013$; Figure 2D). To see if the relationships between LH or FSH and T were mediated by AR-CAGn (i.e., that the inhibitory effect of T depended on LH/FSH depended on AR-CAGn), we looked at the interaction between T and AR-CAGn in the prediction of LH and FSH. In our population, AR-CAGn did not modify the negative feedback effects of plasma T, salivary AM-T, or salivary PM-T for LH or FSH ($P > .30$ for all).

4 | DISCUSSION

We examined the potential role of the AR-CAGn, a highly variable polymorphism in the androgen receptor, in

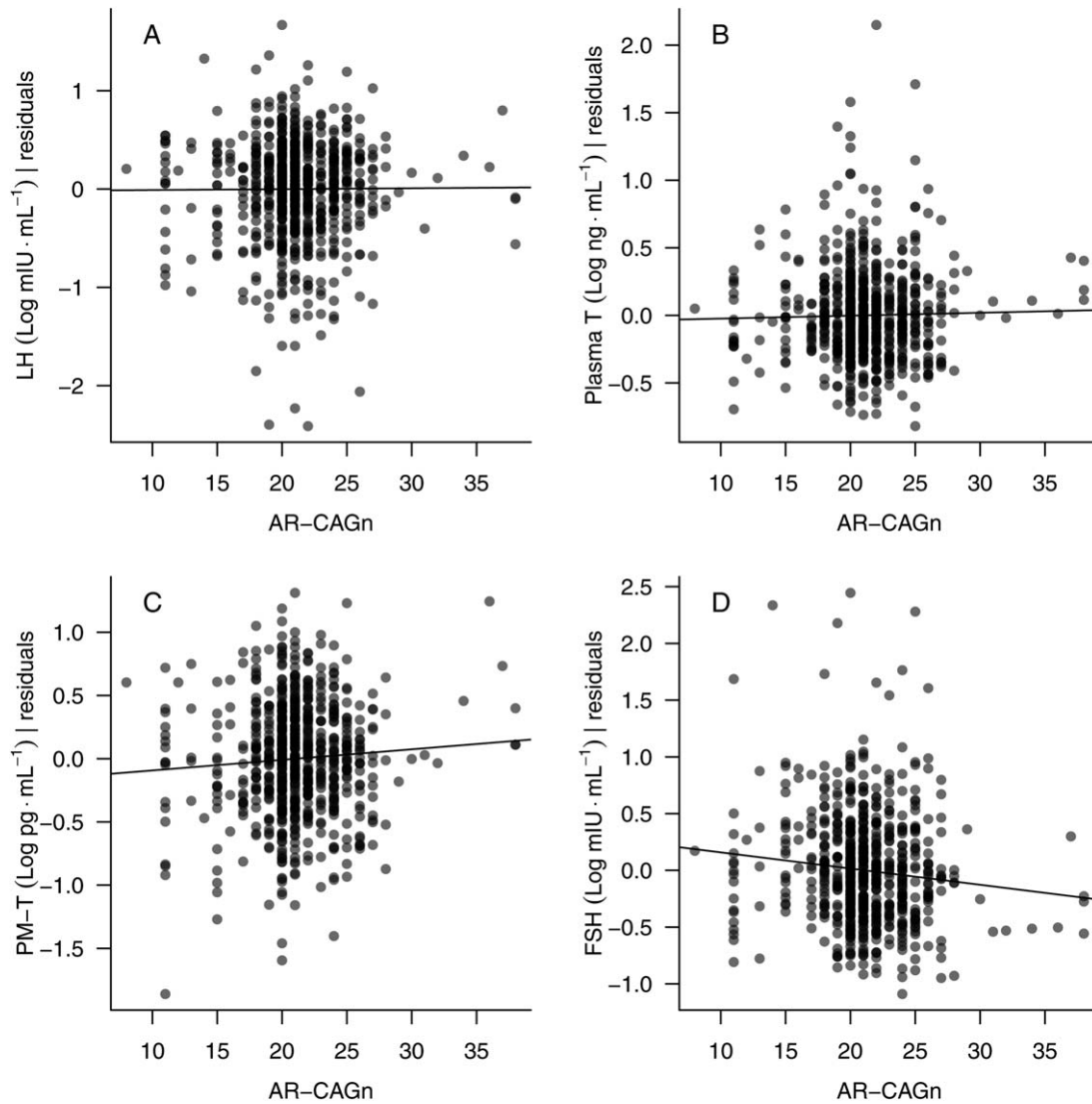


FIGURE 2 Partial correlations between AR-CAGn and plasma LH (A), plasma T (B), salivary PM-T (C), and plasma FSH (D). The relationship between AR-CAGn and PM-T was marginally significant ($P = .066$), while the relationship between plasma AR-CAGn and FSH was highly significant ($P = .005$)

regulating the HPG axis in young, adult Filipino men. Contrary to our predictions, AR-CAGn was a weak predictor of our measures of HPG axis function, showing no relationship with LH, plasma T, or salivary AM-T. We did find evidence

for a statistically borderline positive relationship between AR-CAGn and salivary PM-T, as predicted. Contrary to our predictions, we also found a highly significant negative association between AR-CAGn and circulating levels of FSH,

TABLE 2 Relationship between AR-CAGn and hormones of the HPG axis^a

	β -estimate	Std. Error	<i>P</i> value	η_p^2
Plasma LH (log mIU/mL)	0.001	0.005	.867	< 0.001
Plasma T (log ng/mL)	0.002	0.003	.538	< 0.001
Salivary PM-T (log pg/mL)	0.009	0.005	.066	0.005
Salivary AM-T (log pg/mL)	0.005	0.004	.285	0.002
Plasma FSH (log mIU/mL)	-0.014	0.005	.005**	0.011

^aAll estimates are from models controlling for time of sample and fatherhood status. β -estimates non-standardized—a one unit increase in AR-CAGn is associated with a corresponding (logged) unit change in the dependent variable.

though AR-CAGn only accounted for roughly 1% of the variation in FSH after controlling for fatherhood status and time of sample. These findings point to weak relationships between AR-CAGn and our measures of HPG axis regulation in this sample.

Based on previous work showing that the transcriptional activity of the AR decreases with AR-CAGn length (Buchanan et al., 2004; Kazemi-Esfarjani et al., 1995; Simanainen et al., 2011), we predicted higher LH and T in men with longer AR-CAGn due to an attenuation of the inhibitory effect of T at the level of the hypothalamus and pituitary. Contrary to our predictions, we found only borderline evidence for elevated salivary PM-T among men with longer AR-CAGn, and no relationship between AR-CAGn and total plasma T, salivary AM-T, or LH. The absence of a relationship between AR-CAGn and LH is consistent with several other studies (Huhtaniemi et al., 2009; Krithivas et al., 1999; Van Pottelbergh et al., 2001), and likely reflects the highly pulsatile, and, therefore, “noisy,” nature of this gonadotropic hormone in vivo.

In addition to the current study, several others have reported a weak or absent relationship between the AR-CAGn and circulating T in non-clinical populations (Canale et al., 2005; Goutou et al., 2009; Krithivas et al., 1999; Skjaerpe et al., 2008; Van Pottelbergh et al., 2001). However, the most highly powered studies ($N > 1800$) all report small but consistently positive effects of AR-CAGn on both free and total T (Crabbe et al., 2007; Haring et al., 2012; Huhtaniemi et al., 2009; Lindstrom et al., 2010). The small effect of the AR-CAGn on total T, estimated by Lindstrom et al. (2010) in a population of over 4,500 men, may help explain the marginally significant but positive correlation between PM-T and AR-CAGn we report in our study of 722 young males. A power analysis using the average of the estimated effects from several studies (Crabbe et al., 2007; Haring et al., 2012; Huhtaniemi et al., 2009; Krithivas et al., 1999; Lindstrom et al., 2010; Van Pottelbergh et al., 2001) supported this conclusion; a sample size of roughly 1,000 men would have had an 80% chance of detecting an effect of AR-CAGn on T of this size. In contrast to salivary PM-T, there was no indication of any relationship between AR-CAGn and AM-T. The biological processes underpinning differences between these two measures of salivary T and AR-CAGn are unclear; however, testosterone levels at the time of waking appear distinct from T at other times of day. Shortly after falling asleep, LH surges cause T to rise dramatically, remaining high throughout the night (Luboshitzky, Herer, Levi, Shen-Orr, & Lavie, 1999), and decreasing rapidly upon waking (Kuzawa, Georgiev, McDade, Bechayda, & Gettler, 2015). The factors that allow for the upregulation of the testosterone “set-point” during sleep have not been well described, but imply responses to circadian fluctuations

in other hormones that are at least partly independent of the negative feedback loop in the HPG axis via the AR (Andersen, Alvarenga, Mazaro-Costa, Hachul, & Tufik, 2011; Luboshitzky et al., 1999). None of the large studies mentioned above that report a positive relationship between AR-CAGn and T investigated *waking* testosterone levels, as we have by measuring AM-T. Further work examining the role of AR-CAGn in the regulation of the HPG axis during sleep (and immediately upon waking) will be necessary, therefore, since our results for AM-T can neither confirm nor refute findings during other times of day.

Our measurement of total plasma T includes free, albumin-bound, and SHBG-bound testosterone, while salivary AM-T and PM-T measure only free and albumin-bound or bioavailable T. The relationship between AR-CAGn and bioavailable, salivary PM-T is, therefore, expected to be more pronounced than AR-CAGn and total T, as our data also suggest. Nevertheless, in contrast to our findings, several studies have reported a positive correlation between AR-CAGn and total plasma T (Crabbe et al., 2007; Haring et al., 2012; Huhtaniemi et al., 2009; Lindstrom et al., 2010; Walsh et al., 2005). Compared with our population, which is highly homogeneous with respect to age (21.5 ± 0.5 years), other studies cover a much broader subject age range, including men into their 70s, 80s, and even 90s (Crabbe et al., 2007; Haring et al., 2012; Huhtaniemi et al., 2009; Lindstrom et al., 2010; Walsh et al., 2005). Age-related declines in T are well documented, as are age-related *increases* in SHBG (Morley, 2001). As a result, the relative proportion of bioavailable T in older men is significantly lower (Stanworth and Jones, 2008), and relative to longer AR-CAGn, shorter, more transcriptionally active AR-CAGn appears to be more sensitive to age-related declines in bioavailable T (Chang, 2002). This is consistent with the work of Krithivas and colleagues, who found that rather than predicting total or free testosterone and LH, longer AR-CAGn was associated with a more gradual rate of decline in these hormones over a roughly ten year interval (Krithivas et al., 1999). It is unclear whether or not—and if so to what extent—age can explain the lack of a relationship between AR-CAGn and total T in our study. Nevertheless, the demographic composition of our population relative to others studied merits consideration when interpreting our findings.

LH and FSH are secreted from the pituitary in response to the release of GnRH from the hypothalamus. Given the overlapping role of T in the inhibitory control of LH and FSH at both the hypothalamus and pituitary, we predicted similar relationships between AR-CAGn and both of these hormones. However, since FSH is in part independently regulated through a separate negative feedback loop involving the binding of inhibin B at the hypothalamus, we expected a weaker relationship between AR-CAGn and

FSH. We were somewhat surprised to find such a clear relationship between AR-CAGn and FSH, and intrigued that similar results are reported elsewhere (Huhtaniemi et al., 2009). Data from Huhtaniemi et al. (2009) as well as human experiments (Hayes, DeCruz, Seminara, Boepple, & Crowley, 2001) may shed light on our findings. Circulating T regulates LH secretion a) directly through binding to the AR, and b) indirectly via aromatization of T to estradiol (E_2) (Hayes et al., 2001). In contrast, the effects of T on FSH are mainly *indirect* by way of aromatization of T to E_2 . As a result, longer AR-CAGn is thought to contribute to FSH suppression indirectly, both by reducing the androgenic activity of T and by increasing circulating E_2 levels (Huhtaniemi et al., 2009; Lindstrom et al., 2010). While these conclusions are consistent with the strong negative relationship between AR-CAGn and FSH reported here, other endocrine measures such as E_2 and inhibin B levels from our subjects would be necessary to make any conclusive statements about underlying causation. Indeed, E_2 and inhibin B are central to HPG axis regulation in adult males (Hayes, Seminara, DeCruz, Boepple, & Crowley, 2000), and these data would provide a more complete picture of the effect of AR-CAGn on circulating T, LH, and FSH in our population. Regulatory control of the HPG axis clearly involves multiple components operating in tandem to provide both individual and circadian variation, with the AR-CAGn providing but one piece of the puzzle.

Our findings should be considered in the context of several limitations. First, spermatogenesis is integral to male fertility and reproductive success, but involves both FSH and inhibin B. Without the latter, inferences about spermatogenesis based on the current study cannot be readily made. Second, our measures of HPG axis function were derived from single salivary or plasma measures. For salivary and plasma T, average hormone levels across multiple days would have improved reliability and statistical power (Dabbs, 1990). With greater statistical power, the marginally significant relationship between AR-CAGn and salivary PM-T might have reached statistical significance. However, since LH, plasma T and AM-T did not approach significance, factors other than statistical power are needed to explain our findings. For LH, the most important limitation was likely the highly pulsatile secretion of this hormone, for which a single measurement cannot adequately capture its dynamic nature. However, the fact that LH positively related to both plasma and salivary T in this sample (see also Gettler, Agustin, & Kuzawa, 2010; Kuzawa et al., 2009), as well as the strong relationship between AR-CAGn and FSH that we observed, suggests that our measures succeeded in capturing important and biologically relevant variation in the HPG axis.

Although our findings point to a modest effect of the AR-CAGn polymorphism on HPG axis regulation in young men, the AR-CAGn genotype may still contribute to

phenotypically-relevant traits by moderating the somatic effects of circulating T at target tissues. Numerous behavioral, somatic, and disease-related traits arising from variation in the AR-CAGn have been described (Rajender, Singh, & Thangaraj, 2007; Ryan & Crespi, 2013). For example, inflammation and heart disease (Alevizaki et al., 2003; Gonzalez Hernandez et al., 2008), body mass and adiposity (Gustafson, Wen, & Koppanati, 2003; Pausova et al., 2010; Stanworth, Kapoor, Channer, & Jones, 2008), and psychological health and social behavior (Jönsson et al., 2001; Schneider et al., 2011; Vermeersch, T'Sjoen, Kaufman, Vincke, & Van Houtte, 2010), have all been connected with AR-CAGn, and future analyses in this sample will capitalize on the extensive phenotypic data accompanying these data to explore these possibilities.

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AUTHOR CONTRIBUTIONS

CWK, TWM, DTAE, LTG, and MGH designed the study and directed data collection. MR and DTAE conducted laboratory analysis. CPR, MR, and DTAE carried out laboratory or genetic analysis quality control. CPR and CWK analyzed the data. CPR and CWK wrote the manuscript. TWM, DTAE, LTG, MGH, and MR edited the manuscript for intellectual content.

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