Increased Estrogen Rather Than Decreased Androgen Action Is Associated with Longer Androgen Receptor CAG Repeats

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Context: The individual variability in the waning androgenic-anabolic functions of aging men may be influenced by the CAG repeat polymorphism in exon 1 of the *androgen receptor* (*AR*), affecting androgen sensitivity. However, findings on its phenotypic effects are inconclusive.

Objective: The aim was to investigate the relationships between health status, various reproductive hormones, and the *AR* CAG repeat length.

Design: We conducted a multinational prospective cohort observational study with cross-sectional baseline data.

Setting: This was a population survey of community-dwelling men.

Participants: Men (40–79 yr old; n = 3369) were randomly recruited from centers in eight European countries; CAG repeat analysis was performed in 2878 men.

Main Outcome Measures: We measured the correlations of the CAG repeat length with selected endocrine, metabolic, and phenotypic parameters related to aging and sex hormone action.

Results: Only minor differences were found in CAG repeat lengths between the eight European countries. They showed significant positive association with total, free, and bioavailable levels of testosterone (T) and estradiol. FSH but not LH correlated inversely with CAG repeat length. Significant associations were found with bone ultrasound parameters at the calcaneus. Negative correlation was found with triglycerides, but not with other blood lipids or with anthropometry, blood pressure, hemoglobin, insulin sensitivity, or sexual and prostatic functions.

Conclusions: The *AR* CAG repeat length correlates significantly with serum T and estradiol of aging men. Weaker transcriptional activity of the AR with longer CAG-encoded polyglutamine repeats appears to be totally or nearly totally compensated for by higher T levels. The residual phenotypic correlations may reflect differences in estrogen levels/actions after aromatization of the higher T levels. **(*J Clin Endocrinol Metab* 94: 277–284, 2009)**

Since the original discovery that the length of the CAG repeat polymorphism in exon 1 of the *androgen receptor (AR)* correlates inversely with the strength of androgen action, its associations with phenotypic variations in androgen action have been studied extensively (1). Shorter repeats with stronger androgen action have been associated, for example, with increased risk of prostate cancer, adverse plasma lipid profile, and male-type alopecia. Longer repeats with weaker androgen action have been associated with impaired spermatogenesis. Extreme repeat lengths (>40) cause spinobulbar muscular atrophy (Kennedy's syndrome), including a variable degree of androgen insensitivity.

When the hypothalamic-pituitary-gonadal axis functions normally, one could expect that the diminished testosterone (T) feedback associated with weakened AR activity would be compensated for by increased LH-stimulated androgen production. We could hypothesize that the compensation is incomplete if signs of androgen deficiency occur with longer CAG repeats. However, closer scrutiny of existing data does not reveal definite signs of androgen deficiency in men with longer repeats (1, 2). Several (2, 3), but not all (4–7), studies have shown that the CAG repeat length directly correlates with serum T levels, indicating that the weaker AR activity may indeed be compensated for by higher androgen levels. In addition, some (2, 7), but not all (5, 6), studies have documented concurrent increases in estrogen levels. The discrepant findings on CAG repeat *vs.* sex hormone levels are at least partly due to small sample sizes, but different genetic backgrounds and modifying inputs between populations may also contribute to the variability. The functional importance of the enhanced estrogen production and altered androgen/estrogen balance with increased CAG repeat lengths has received little attention. Pointers to its importance do exist, such as gynecomastia (8, 9) and reduced risk for male-type alopecia (10) in patients with Kennedy's syndrome. However, most studies have interpreted the phenotypic effects of long CAG repeats to be caused by androgen deficiency. Using observations of the unique European Male Ageing Study, comprising *AR* CAG repeat lengths and endocrine and clinical characteristics of nearly 3000 men, aged 40–79 yr, we provide evidence that the direct correlation of the *AR* CAG repeat length with estrogen, rather than T, may be important in determining the phenotypic effects of this polymorphism.

Subjects and Methods

Study population

The European Male Ageing Study (EMAS), as described elsewhere (11–13), is a European Union-funded multicenter, prospective, population-based study of determinants of male aging. The eight participating centers are: Florence (Italy), Leuven (Belgium), Lodz (Poland), Malmö (Sweden), Manchester (United Kingdom), Santiago de Compostela (Spain), Szeged (Hungary), and Tartu (Estonia). We present here data from the first phase of the study, a cross-sectional survey of a random population sample of men aged 40–79 yr, completed in 2003–2005. Ethics approval for the study was obtained in each center according to local requirements. The number of men recruited ranged from 396 to 451 per center (total n = 3369). DNA extraction and CAG repeat analysis was carried out on 294 to 407 samples per center (total n = 2878). There was no difference in age, demographics, hormone, or bone ultrasound findings between men with DNA available and unavailable (data not shown).

Blood sampling and processing

A single morning sample of fasting venous blood (before 1000 h), processed and stored according to standard protocols, was used for the DNA, hormone, hematological, and biochemical measurements. DNA was extracted from leukocytes using standard phenol:chloroform extraction after differential lysis of erythrocytes. Purified DNA was precipitated and stored at −80 C until analyzed.

CAG repeat determination

Genotyping of the CAG repeat was carried out in the laboratory of the Centre for Integrated Genomic Medical Research (The University of Manchester), using fluorescently-labeled PCR. Ten nanograms of DNA were amplified in 10-μl reactions containing 2.5 pmol each of fluorescently labeled forward and reverse primer, 10 × PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, and 0.2 U *Taq* DNA polymerase. The primer sequences were: forward, 5′-TCC AGA ATC TGT TCC AGA GCG TGC-3′; and reverse, 5′-GCT GTG AAG GTT GCT GTT CCT CAT-3′. Reactions were cycled at 95 C for 5 min; 10 cycles of 94 C for 10 sec, 55 C for 30 sec, and 72 C for 30 sec; 20 cycles of 89 C for 20 sec, 55 C for 30 sec, and 72 C for 30 sec; and finally, 72 C for 10 min. Samples were then run on an ABIPRISM 3100 Genetic Analyser (Applied Biosystems, Foster City, CA) and genotyped using Genescan (Applied Biosystems). Allele frequencies were checked for consistency with HapMap data or literature where possible.

Hormone assays

The serum samples were transported in frozen state to the General Laboratory, Azienda Ospedaliero-Universitaria Careggi (Florence, Italy), where they were assayed for T, estradiol (E2), FSH, LH, and SHBG by the Modular E170 platform electrochemiluminescence immunoassays (Roche Diagnostics, Mannheim, Germany) as described previously (13). Free T and E2 levels were derived from total hormone, SHBG, and albumin concentrations (14). The details of assay performance and quality control have been presented before (Refs. 11 and 13; also see supplementary material, published as supplemental data on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

Anthropometric parameters

Body weight, height, body mass index (BMI), and body circumferences (waist, hip, midcalf, and mid-upper arm) were measured as described before (11). Percentage of body fat was calculated from total body density (15).

Quantitative ultrasound (QUS)

All subjects had ultrasound assessment of the nondominant calcaneus using a Sahara Clinical Sonometer (Hologic, Bedford, MA). Measured parameters included the velocity of ultrasound transmission through bone [speed of sound (SOS) in meters per second from the sound

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Abbreviations: AR, Androgen receptor; BMD, bone mineral density; BMI, body mass index; BUA, broadband ultrasound attenuation; CI, confidence interval; E2, estradiol; EMAS, European Male Ageing Study; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; HOMA-B, HOMA pancreatic β-cell function; HOMA-IR, HOMA insulin resistance; HOMA-S, HOMA sensitivity; IPSS, International Prostate Symptom Score; LDL, low-density lipoprotein; PSA, prostate-specific antigen; QUI, QUS index; QUICKI, quantitative insulin sensitivity check index; QUS, quantitative ultrasound; SOS, speed of sound; T, testosterone.

propagation time between the transducers] and the rate of loss of ultrasonic intensity with frequency [broadband ultrasound attenuation (BUA) in decibels per megahertz using Fourier transformation of the recorded signal]. Additional machine-derived parameters were QUS-estimated bone density in grams per square centimeter [bone mineral density (BMD) = 0.002592 × (BUA + SOS) − 3.687] and QUS index (QUI), a measure of stiffness [QUI = 0.41 (SOS) + 0.41 (BUA) − 571]. Short-term precision of the method was established on duplicate measurements performed in 20 randomly selected cohort members in the Leuven center. The *in vivo* coefficients of variation were 2.8 and 0.3% for BUA and SOS, respectively, and 2.3 and 3.4% for QUI and QUS-estimated bone density, respectively. Further technical details about the BMD measurements and their quality control are presented elsewhere (11).

Blood hematology, biochemistry, and lipids

A number of routine hematological and biochemical markers of general health were measured using standardized methods under good laboratory practice conditions in internationally and/or nationally accredited hospital laboratories in each of the centers (11). Hematology analyses included hemoglobin, and platelet, and red and white cell counts. Biochemical measurements included glucose, albumin, cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, and low-density lipoprotein (LDL) cholesterol (calculated from total and HDL cholesterol, and fasting triglycerides using the Friedewald equation (16).

Parameters of insulin sensitivity

Insulin was measured in a single laboratory (Complejo Hospitalario Universitario de Santiago, Santiago de Compostela, Spain) using a specific ELISA assay (17). Insulin resistance, sensitivity, and pancreatic β-cell function were estimated according to the homeostasis model assessment (HOMA-IR, HOMA-S, and HOMA-B, respectively) (18). In addition, the quantitative insulin sensitivity check index (QUICKI) was calculated (19).

Sexual and prostate function

Subjects completed a sexual function questionnaire (12), including questions about the frequency of morning erections and the ability to maintain an erection, and the Beck Depression Inventory questionnaire (20), including a question concerning loss of interest in sex. Prostate function was assessed by measurements of serum PSA and completion of the International Prostate Symptom Score (IPSS) questionnaire (21).

Statistical analyses

In the analysis, outcomes such as hormone levels, QUS parameters, and PSA/IPSS levels were treated as continuous variables. Sexual dysfunction measures were dichotomized: poor morning erection was defined as the frequency of morning erections in the last 4 wk: none/less than one per month *vs.* two to three per month or more. Erectile dysfunction was defined as the ability to reach and keep an erection good enough for sexual intercourse in the last 4 wk: never/sometimes *vs.* usually/always (12). Poor libido was defined as loss of interest in sex in the last 4 wk: completely/much less interested *vs.* less interested/no change. *AR* CAG repeat length was treated as a continuous variable.

Descriptive statistics were used to characterize the distribution of CAG repeat length by age and center. The association between endocrine and anthropometric, bone, IPPS, and PSA measures and repeat length was assessed initially using scatter plots, superimposing linear lines and lowess (LOcally-WEighted Scatter plot Smooth) curves (22). Linear regression was then used to determine the association between each of the endocrine factors and repeat length, with the endocrine factors as the dependent variables. The results are expressed as absolute differences (β coefficients) and 95% confidence intervals (CI). Logistic regression was used to determine the association between the sexual dysfunction categories and repeat length, with results expressed as odd ratios and 95% CI compared with a reference (usually no or minimal dysfunction). Adjustments were made for age and center, calculating corrected SE values to account for differences between centers. Statistical analysis was performed using STATA version 9.2 (http://www.stata.com). All the statistically significant associations remained significant after exclusion of outliers in CAG length.

Results

AR CAG repeat lengths in the European populations

The characteristics of the 2878 men included in the analysis are shown in Table 1. The mean (± SD) repeat length was 22.1 ± 3.1. There were some small, albeit statistically significant, population differences (n = 294–407 per center) (Table 2), the largest, 0.6, being between the mean CAG repeat length of men from Leuven, Belgium (21.8) and Lodz, Poland (22.4) ($P < 0.05$). A standardized normal probability plot (data not shown) indicated that the repeat length was normally distributed. There was no association between repeat length and age (Table 3 and supplemental data).

CAG repeat length and circulating T, E2, and SHBG levels

Men with longer CAG repeat lengths had significantly ($P < 0.001$) higher levels of total T (Fig. 1A and Table 4), an association that persisted after adjustment for age and center (β coefficient = 0.134 nmol/liter; 95% CI, 0.067, 0.201). Similar results were seen for free and bioavailable T (β coefficient = 2.643 pmol/liter; 95% CI, 1.688, 3.598; and β coefficient = 0.065

TABLE 1. Characteristics of subjects (n = 2878)

	Mean (SD) or %
Age (yr)	59.9 (11.1)
Height (m)	1.73 (0.07)
Weight (kg)	83.3 (14.0)
Total T (nmol/liter)	16.5 (5.9)
Free T (pmol/liter)	291.7 (92.5)
Bioavailable T (nmol/liter)	7.0 (2.3)
Total E2 (pmol/liter)	92.5 (29.0)
Free E2 (pmol/liter)	1.6 (0.5)
Bioavailable E2 (pmol/liter)	64.5 (20.8)
FSH (nmol/liter)	8.6 (8.9)
LH (nmol/liter)	6.2 (4.3)
SHBG (nmol/liter)	42.8 (19.7)
PSA (μg/liter)	1.9 (4.9)
IPSS (0–35)	5.7 (6.0)
BUA (dB/MHz)	80.1 (18.9)
SOS (m/sec)	1550.5 (33.9)
BMD (g/cm^2)	0.54 (0.14)
QUI	97.8 (21.8)
Poor morning erection[a]	39.1%
Erectile dysfunction[b]	29.9%
Poor libido[c]	14.4%

[a] Poor morning erection is defined as the frequency of morning erections in the last 4 wk: none/< 1 per month *vs.* 2–3 per month or more.

[b] Erectile dysfunction is defined as the ability to get and keep an erection that would be good enough for sexual intercourse in the last 4 wk: never/sometimes *vs.* usually/always.

[c] Poor libido is defined as loss of interest in sex in the last 2 wk: completely/much less interested *vs.* less interested/no change.

TABLE 2. *AR* CAG repeat lengths in the different centers of the EMAS study, in descending order of length

Center	n	Mean	SD	Median
Lodz	393	22.4	3.3	22
Florence	407	22.3	3.0	22
Tartu	294	22.3	3.0	22
Szeged	383	22.2	3.3	22
Malmö	308	22.0	3.2	22
Santiago	369	22.0	3.1	21
Manchester	370	21.9	3.0	21
Leuven	354	21.8	3.0	21
Overall	2878	22.1	3.1	22

nmol/liter; 95% CI, 0.041, 0.089, respectively). In addition, there was a positive linear relationship between repeat length and the product of T × LH, an index reflecting relative androgen insensitivity (β coefficient = 0.802; 95% CI, 0.010, 1.594).

The association between CAG repeat length and E2 was very similar to that with T, showing a significant ($P < 0.001$) positive association between total E2 levels and repeat length (Fig. 1B and Table 4). It persisted after adjustment for age and center (β coefficient = 0.656 pmol/liter; 95% CI, 0.335, 0.977). Similar results were seen for free E2 (β coefficient = 0.012 pmol/liter; 95% CI, 0.006, 0.018) and bioavailable E2 (β coefficient = 0.486 pmol/liter; 95% CI, 0.254, 0.718). As expected, total T and E2 levels showed a clear linear correlation (β coefficient = 0.075; $P < 0.001$) (Fig. 1C). Furthermore, there was no association between CAG length and E2/T ratio (β coefficient = 0.001; 95% CI, −0.039, 0.042), suggesting that the same proportion of T was aromatized to E2 at the entire range of CAG repeat lengths.

TABLE 3. Parameters in which nonsignificant correlations with the *AR* CAR repeat length were found in the study population

Parameter	β coefficient	95% CI
Age	−0.001 yr	−0.011, 0.009
Height	−0.064 cm	−0.139, 0.010
Weight	−0.054 kg	−0.209, 0.102
Waist circumference	0.031 cm	−0.093, 0.155
Waist/hip ratio	0.000	−0.001, 0.001
% Body fat	0.000	0.000, 0.001
Total cholesterol	−0.007 mmol/liter	−0.020, 0.006
HDL-cholesterol	0.003 mmol/liter	−0.001, 0.007
LDL-cholesterol	−0.004 mmol/liter	−0.015, 0.007
HOMA-IR	−0.002	−0.038, 0.034
HOMA-S	0.000	−0.004, 0.004
HOMA-B	−0.093	−1.157, 0.970
QUICKI	0.000	−0.001, 0.001
Systolic BP	0.028 mm Hg	−0.192, 0.248
Diastolic BP	0.011 mm Hg	−0.148, 0.126
Hemoglobin	0.002 g/liter	−0.010, 0.014
Platelets	0.221 (× 10^{-6}/liter)	−0.415, 0.856
Red blood cell count	0.002 (× 10^{-6}/liter)	−0.002, 0.007
White blood cell count	−0.017 (× 10^{-6}/liter)	−0.036, 0.003
PSA	0.005 μg/liter	−0.032, 0.042
IPSS prostatic symptoms questionnaire	−0.034	−0.100, 0.033
	Odds ratio	
Poor morning erections	0.989	0.963, 1.016
Erectile dysfunction	0.990	0.960, 1.021
Poor libido	0.995	0.959, 1.033

BP, Blood pressure.

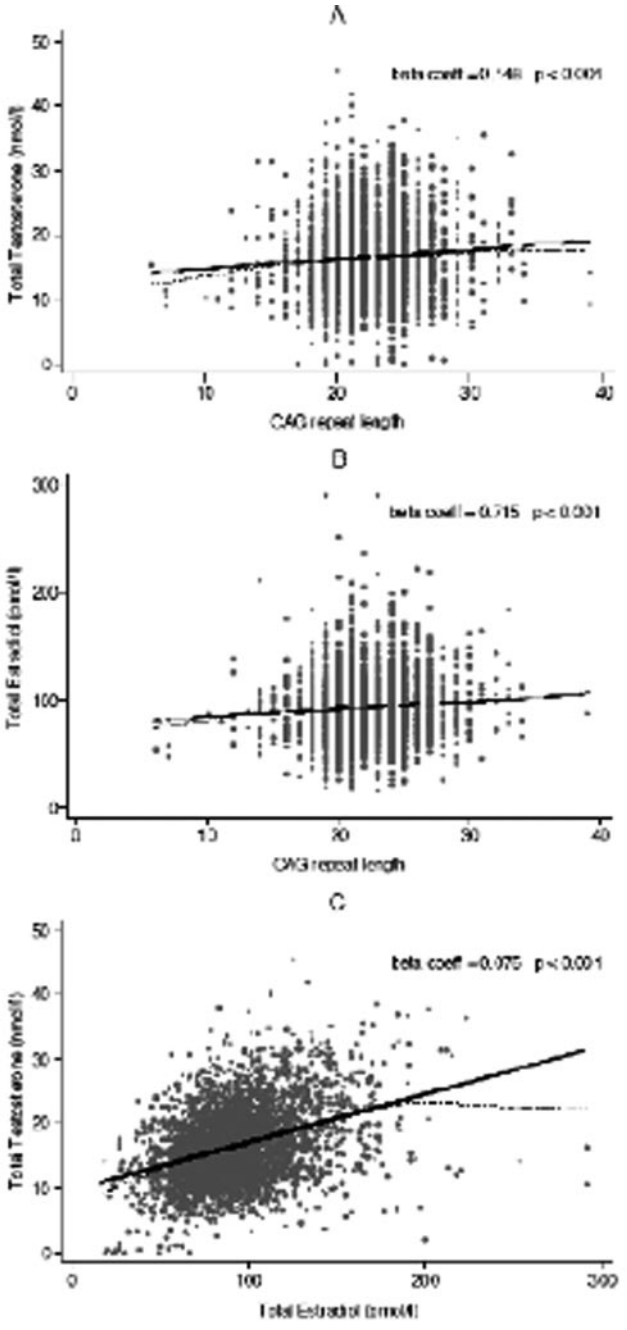

FIG. 1. Influence of *AR* CAG repeat length on total T (A) and E2 (B) levels, and the correlation of serum T and E2 levels (C). The *solid lines* represent the continuous relationship, the *dashed lines* represent locally weighted scatterplot smoothing (lowess). β coeff is the coefficient from linear regression.

Repeat length was weakly and negatively associated ($P < 0.05$) with serum FSH levels (β coefficient = −0.110 U/liter; 95% CI, −0.207, −0.013) (Table 4). The FSH/LH ratio also decreased with increasing repeat length (β coefficient = −0.020; 95% CI, −0.035, −0.004), but there was no relationship between LH levels and CAG repeats (β coefficient = −0.008 U/liter; 95% CI, −0.056, 0.039). Likewise, SHBG levels were not associated with CAG repeat lengths (β coefficient = 0.004 nmol/liter; 95% CI, −0.206, 0.213).

TABLE 4. Influence of CAG repeat length on sex hormones, gonadotropins, SHBG, and calcaneal ultrasound parameters

	Independent variable: CAG repeat length (6–39 repeats)	
Dependent variables	**β coefficient (95% CI)**	***P* value**
Sex hormones		
Total T (nmol/liter)	0.134 (0.067, 0.201)	<0.001
Free T (pmol/liter)	2.643 (1.688, 3.598)	<0.001
Bioavailable T (nmol/liter)	0.065 (0.041, 0.089)	<0.001
T × LH product	0.802 (0.010, 1.594)	0.047
Total E2 (pmol/liter)	0.656 (0.335, 0.977)	<0.001
Free E2 (pmol/liter)	0.012 (0.006, 0.018)	<0.001
Bioavailable E2 (pmol/liter)	0.486 (0.254, 0.718)	<0.001
E2/T ratio	0.001 (−0.039, 0.042)	0.95
Gonadotropins and SHBG		
FSH (nmol/liter)	−0.110 (−0.207, −0.013)	0.03
LH (nmol/liter)	−0.008 (−0.056, 0.039)	0.73
FSH / LH ratio	−0.020 (−0.035, −0.004)	0.01
SHBG (nmol/liter)	0.004 (−0.206, 0.213)	0.97
Calcaneal ultrasound		
QUS-estimated bone density (g/cm^2)	0.002 (0.000, 0.003)	0.02
BUA (db/Mhz)	0.123 (−0.093, 0.339)	0.26
SOS (m/s)	0.420 (0.037, 0.803)	0.03
QUI	0.279 (0.029, 0.528)	0.03

The sex hormones, gonadotropins, and ultrasound parameters are dependent variables, and CAG repeat length is the independent variable adjusted for age and center. For example, total T level increases by 0.134 nmol/liter for every one unit increase in repeat length.

CAG length and anthropometric parameters

There was no association between repeat length and any of the anthropometric measures, including height, weight, waist circumference, waist/hip ratio, and percentage of body fat (Table 3).

CAG length and QUS parameters

Repeat length was positively associated with ultrasound parameters at the calcaneus (Table 4). Increasing repeat length was significantly associated with increasing SOS (β coefficient = 0.420 m/sec; 95% CI, 0.037, 0.803) and the derived parameters, QUS-estimated bone density (β coefficient = 0.002 g/cm^2; 95% CI, 0.000, 0.003) and QUI (β coefficient = 0.279; 95% CI, 0.029, 0.528). There was, however, no association with BUA (β coefficient = 0.123 dB/MHz; 95% CI, −0.093, 0.339).

The associations between CAG length and the QUS parameters became slightly weaker and nonsignificant after further adjustment for E2: SOS (β coefficient = 0.332 m/sec; 95% CI, −0.051, 0.716; $P = 0.09$), QUS-estimated bone density (β coefficient = 0.001 g/cm^2; 95% CI, −0.0002, 0.003; $P = 0.08$), QUI (β coefficient = 0.217; 95% CI, −0.033, 0.466; $P = 0.09$), and BUA (β coefficient = 0.072 dB/MHz; 95% CI, −0.144, 0.288; $P = 0.52$).

CAG length and metabolic, cardiovascular, and hematological parameters

With respect to blood lipids, a weak negative association ($P < 0.05$) was found between CAG length and triglycerides (β coefficient = −0.015 mmol/liter; 95% CI, −0.027, −0.003), but no associations were observed with total cholesterol, HDL-cholesterol, or LDL-cholesterol. There were no associations with the measures of insulin sensitivity/resistance, as monitored by HOMA-IR, HOMA-S, HOMA-B, or QUICKI. Similarly, there was no correlation between CAG repeat length and systolic or diastolic blood pressure, nor did we find associations between CAG repeat length and the hematological parameters, including hemoglobin, and platelet and red and white blood cell counts (Table 3). BMI did not influence the relationships between CAG length and plasma lipids, blood pressure, or measures of insulin resistance (results not shown). Hence, BMI would be unlikely to confound any of the other relationships we have presented.

CAG length and measures of sexual and prostatic function

There was no difference in repeat lengths between men with and without sexual dysfunction as defined by poor morning erection, erectile dysfunction, and poor libido. Similarly, there was no association between repeat length and serum PSA levels or the IPSS prostatic symptoms questionnaire (Table 3).

Discussion

We present here the largest dataset to date of *AR* CAG repeat measurements from a group of 2878 European men aged 40–79 yr. The mean repeat length measured, 22.1 ± 3.1 (SD), is in agreement with previous data on Caucasian populations (1, 23). The availability of extensive clinical and laboratory data allowed detailed and robust genotype/phenotype correlations on the putative effects of this genetic polymorphism of androgen action. Some clear conclusions could be made: 1) The *AR* CAG repeat length correlated directly with all measures (total, bioavailable, free) of serum T and E2, and there was a strong positive correlation between circulating levels of T and E2. 2) Because the transcriptional activity of AR decreases with increasing CAG repeat length (1), a parallel increase in molar concentrations of E2 and T levels implies a concomitant increase in the ratio of estrogen/androgen bioactivity (discussed below). 3) The CAG length correlated positively with calcaneal QUS-estimated bone density and inversely with FSH and FSH/LH ratio (discussed below), lending support to heightened estrogen action in the men with longer repeats. 4) No symptoms or signs suggestive of androgen deficiency were found to correlate with CAG repeat length. Hence, our data suggest that the concomitant increase of circulating T levels in men with longer repeats can adequately compensate for the lower AR activity to prevent apparent deficiency of androgen action. Instead, signs of the simultaneously elevated estrogen action can be detected in the form of increased calcaneal ultrasound parameters and reduced FSH/LH ratio. All expected correlations of CAG repeat length with estrogenicity (*e.g.* SHBG and HDL-cholesterol) did not reach significance, apparently due to the mild nature of associations.

Many publications indicate inverse relations between the repeat length and androgen-dependent clinical endpoints (1). For example, direct correlation of the repeat length has been reported with HDL-cholesterol and flow-mediated arterial dilatation (5,

24), as well as body fat, insulin, and leptin (25). Inverse correlations have also been detected with acne and male-type balding (26, 27), sperm counts (28, 29), BMD (30), as well as prostate size and the serum PSA level (31, 32). However, these findings are equaled or outnumbered by reports of equivocal, or even opposite associations, as extensively reviewed recently by Rajender *et al.* (1). Uncertainty in particular applies to the two most extensively studied CAG repeat associations, *i.e.* to male infertility and prostatic cancer. Thus, although the weaker activity of AR with longer CAG repeats is well documented, it remains unclear to what extent the increased androgen levels can compensate for this. Admittedly, the recent meta-analyses show significant correlation of the CAG repeat length with idiopathic male infertility (23) and marginally inverse association with prostate cancer risk (32). Reasons for the inconsistency of findings include small numbers of subjects, type of subjects studied (patients *vs.* general population), effect of race/ethnicity, and inconsistencies in inclusion criteria. EMAS avoids some of these drawbacks with its large sample size randomly selected from the general population and its comprehensive and carefully standardized data.

The *AR* CAG repeat length correlated significantly not only with the total, free, and bioavailable levels of serum T but also with E2. Similar T/CAG correlation has been shown in some (2, 3), but not all (4–7, 30), previous reports. Two studies have shown that the CAG length and T correlate only in older men (3, 4), but we found no interaction of age with the positive correlation of T levels and the CAG repeat length. The correlation of E2 levels with CAG repeats has previously been found to be inconsistent (2, 5–7). We found that the T and E2 concentrations were highly correlated, and hence the E2/T ratio remained constant as the CAG repeat length increased. In this sense, the long CAG repeat with increased E2 production resembles mild forms of androgen insensitivity syndrome (33, 34). Because androgen action is relatively impaired but estrogen action is maintained, when the CAG repeats become longer, the effective bioactive estrogen/androgen ratio must increase. We may therefore question whether any of the reported phenotypic effects of the CAG repeat length are actually due to differences in androgen, but rather in estrogen action. We do not dispute the weaker activity of the AR with long CAG repeats because increasing repeat length significantly associated with elevated T × LH product, as an index of lower androgen sensitivity (2, 33, 34).

The CAG/phenotype correlates can be classified into purely androgenic (such as libido, muscle mass), predominately estrogenic (*e.g.* BMD), and those with opposite effects of estrogen and androgen (*e.g.* spermatogenesis). We did not find any evidence for an inverse relationship of CAG repeat length and the level of androgen action, *i.e.* sexual function, body composition, blood lipids, insulin sensitivity, and hemoglobin. In contrast, there was a significant positive correlation with calcaneal ultrasound parameters, which likely reflects action of the increased E2 levels. Likewise, the suppressed FSH levels in the face of unaltered LH can be considered to represent a relative increase in estrogen action, known to mediate the negative feedback action of steroids on FSH secretion (35). In contrast, both direct actions of estrogen and androgen participate in the feedback regulation of LH, and in the absence of enhanced androgen action with longer CAG repeats the enhanced estrogen action may have been insufficient to achieve significant suppression of LH secretion. Moreover, the documented effects of CAG repeat length on spermatogenesis and prostate cancer risk may reflect differences in the E2/T ratio. Estrogens have direct inhibitory effects on testicular Leydig cell function (36) and spermatogenesis (37), which may explain why men with longer CAG repeats have higher frequency of idiopathic infertility. The role of estrogens in the putative connection between the CAG repeat length and prostate cancer seems less clear because the majority of findings suggest positive associations between estrogen and prostate cancer (38).

Data on effects of *AR* CAG repeat length on BMD of men are confusing. Although most studies show no association in younger or older men (6, 39–41) (n = 91–273), one large study (n = 611) demonstrates a direct correlation between the repeat length and dual-energy x-ray absorptiometry BMD in lumbar spine, femoral neck, and total hip (42), and one small study (n = 110), using the less well-validated end-point of finger ultrasound, shows negative correlation (30). We have shown that ultrasound parameters of the calcaneus correlate positively with the CAG repeat length. Because we also showed positive correlation between CAG repeats with E2, our findings suggest that the elevated estrogen levels in men with longer repeats may lead to higher bone ultrasound parameters. Accordingly, significance of the CAG repeat/BMD correlation was lost when adjusted for E2.

Why only one previous study has shown similar positive correlation while most others showed none or a negative relationship is unclear and may reflect the small sample sizes and differences in bone measurements. Given that variations in *AR* CAG repeats are intrinsically compensated by an intact hypothalamic-pituitary-testicular axis and do not generally result in overt relative androgen deficiency, it is likely that any differences are subtle and detectable only when large populations are analyzed.

Previous studies have observed that CAG repeat length correlates negatively with BMI (43) or positively with fat free mass (4) and HDL-cholesterol (5, 24). However, also discrepant findings exist, showing positive correlation of CAG length with BMI or body fat content (7, 25). A more complicated relationship was found in another study, where unfavorable effects of low T levels on body composition were stronger among men with longer CAG repeats (44). We found no relationship with anthropometric measures, and the CAG/hormone relationships were similar in obese and nonobese men. The only significant correlation in these parameters found in the present study was the inverse correlation of CAG repeat length with triglycerides.

A similar confusion seems to prevail in cardiovascular endpoints. Men with shorter repeats have been found in one study to have more severe coronary artery disease (7), but another study did not confirm this (24). A recent longitudinal report from the Massachusetts Male Aging Study showed no relationship between the CAG repeat length and BMI, HDL-cholesterol, LDL-cholesterol, waist-to-hip ratio, or the prevalence of coronary heart disease (45). Further confusion is added by the unconfirmed finding that the CAG length correlates directly with HDL-cholesterol and flow-mediated vasodilation (5), especially because the same authors subsequently reported direct correla-

tion of CAG with high BMI (25). Our findings did not support association of the CAG repeat with variables considered predictive of cardiovascular diseases. Neither did the repeat length correlate with age, suggesting that its effect on health status of the men was not large enough to affect mortality.

Although all laboratories in the EMAS centers (all major university hospitals) employed internationally standardized methodologies and subscribed to external quality assurance scheme monitoring, it is acknowledged that the possibility of small interlaboratory differences in biochemistry results cannot be completely excluded. We only assessed phenotypic correlations of one *AR* polymorphism in this study, and other alterations of AR structure and function are likely to have additional phenotypic effects.

Taken together, men with longer *AR* CAG repeats had higher T levels, which could compensate partly or totally for the weaker activity of their AR. This is a highly plausible explanation for the lack of any clear signs of androgen deficiency in the EMAS men with longer CAG repeats. A potentially more important finding was the higher E2 levels in men with longer repeats, which paralleled with phenotypic effects indicative of elevated estrogen action. Hence, our current findings suggest that the increased estrogen action and increased estrogen/androgen ratio in association with longer *AR* CAG repeats is paradoxically the main determinant of phenotypic effects of this polymorphism, rather than an androgen-AR effect.

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