The androgen receptor gene CAG repeat in relation to 4-year changes in androgen-sensitive endpoints in community-dwelling older European men

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Abstract

Context: The androgen receptor (AR) gene exon 1 CAG repeat length has been proposed to be a determinant of between-individual variations in androgen action in target tissues, which might regulate phenotypic differences of human ageing. However, findings on its phenotypic effects are inconclusive.

Objective: To assess whether the AR CAG repeat length is associated with longitudinal changes in endpoints that are influenced by testosterone (T) levels in middle-aged and elderly European men.

Design: Multinational European observational prospective cohort study.

Participants: A total of 1887 men (mean $\pm$ s.d. age: 63 ± 11 years; median follow up: 4.3 years) from centres of eight European countries comprised the analysis sample after exclusion of those with diagnosed diseases of the hypothalamic–pituitary–testicular (HPT) axis.

Main outcome measures: Longitudinal associations between the AR CAG repeat and changes in androgen-sensitive endpoints (ASEs) and medical conditions were assessed using regression analysis adjusting for age and centre. The AR CAG repeat length was treated as both a continuous and a categorical (6–20; 21–23; 24–39 repeats) predictor. Additional analysis investigated whether results were independent of baseline T or oestradiol (E₂) levels.

Results: The AR CAG repeat, when used as a continuous or a categorical predictor, was not associated with longitudinal changes in ASEs or medical conditions after adjustments. These results were independent of T and E₂ levels.

Conclusion: Within a 4-year time frame, variations in the AR CAG repeat do not contribute to the rate of phenotypic ageing, over and above, which might be associated with the age-related decline in T levels.

*European Journal of
Endocrinology*
(2016) **175**, 583–593

Introduction

The length of the androgen receptor (AR) tri-nucleotide CAG repeat in exon 1, encoding a polyglutamine tract, has been proposed to regulate androgen action in target tissue. An inverse association between the AR CAG repeat length and androgen action may exist. The AR CAG repeat might regulate androgen action in response to testosterone (T) and dihydrotestosterone (1, 2, 3, 4) in target tissues and affect androgen-sensitive endpoints (ASEs), such as body composition and metabolic parameters (leptin and insulin levels) (5), cardiovascular risk factors (HDL-cholesterol and arterial vasoreactivity) (6), bone density (7) and treatment response to T supplementation (8).

Previously, results from the Massachusetts Male Aging Study (MMAS) have indicated that shorter AR CAG repeats are associated with a greater decline in T levels over time (9). Others have indicated that the presence of either extreme short or long AR CAG repeat (<9 or ≥38) length is associated with increased risk for prostate cancer and Kennedy's disease respectively (4, 10, 11, 12, 13, 14). In addition, Nenonen and colleagues (15) reported a non-linear association between the AR CAG repeat length and fertility status across 33 studies, whereby men with either <22 or >23 CAG repeats were at an increased risk of reduced fertility.

In contrast, Van Pottelbergh and co-workers did not observe an association between the AR CAG repeat and androgen levels, androgen insensitivity index (LH × TT product) or bone markers within a cross-sectional cohort study consisting of ambulatory elderly men (16). In addition, Bentmar-Holgersson and co-workers (17) did not observe an association between the AR CAG repeat and PSA levels or prostate cancer risk within cross-sectional data from the European Male Ageing Study (EMAS). However, additional cross-sectional results from EMAS have led Huhtaniemi and colleagues (18) to propose that the potential downstream consequences of longer AR CAG repeat length and the concomitant decreased androgen action may be modified by compensatory increased oestradiol (E₂) levels. However, most previous studies have been cross-sectional in design or were performed within single centres and hence do not allow for assessment of longitudinal changes and may

have limited external validity. The potential importance of androgen action in ageing men remains unclear. Clinical features developing with ageing may at least in part be a consequence of the age-related decline in T levels modified by variations in tissue response to androgens. Longitudinal cohort studies may provide the opportunity to discern how genetic markers, such as the AR CAG repeat, are related to changes in features of ageing, which are believed to be regulated by androgen action.

The aim of this study is to assess whether the AR CAG repeat length is associated with changes in ASEs, independent of circulating T or E₂ levels, in community-dwelling middle-aged and elderly European men. In addition, longitudinal associations between the AR CAG repeat length and the development of medical conditions, common in the elderly, were assessed in a similar manner.

We hypothesized that the AR CAG repeat length is associated with longitudinal changes in some ASEs that may contribute to the phenotype of ageing men.

Methods

Participants and study design

The EMAS, as described elsewhere (19, 20, 21), is a multi-centre, prospective, population-based cohort study of the endocrine and metabolic determinants of male ageing. The eight participating centres are Florence (Italy), Leuven (Belgium), Lodz (Poland), Malmö (Sweden), Manchester (United Kingdom), Santiago de Compostela (Spain), Szeged (Hungary) and Tartu (Estonia). Ethics approval for the study was obtained in each centre according to local requirements. The number of men recruited ranged from 396 to 451 per centre (total *n*=3369). DNA extraction and AR CAG repeat analysis were carried out on 267–368 samples per centre (total *n*=2659). The protocols used for blood processing and sampling, DNA extraction and determination of AR CAG repeat length within EMAS have been described previously (18). The protocols used for assessment of body composition (lean and fat mass); ultrasound of the heel; blood pressure; haematological,

biochemical, lipid and carbohydrate metabolism; sexual, physical, psychological and prostate function; vitality; and cognitive function in EMAS have been described previously (18, 19, 20). Follow-up assessment was performed a median of 4.3 years (95% CI: 4.23–4.36 years) after the baseline assessment using the same protocols as used in the baseline assessment.

Exclusion criteria

As depicted in Fig. 1, participants (of the total $n=3369$) were excluded if they reported treatment for pituitary, testicular or adrenal disorders and/or use of medication affecting hypothalamic–pituitary–testicular (HPT) axis function at baseline ($n=179$) or follow up ($n=132$). Participants were also excluded if they died ($n=168$), were lost to follow up ($n=407$), missing total T data ($n=78$), if their genotyping failed quality control standards ($n=177$) or if missing AR CAG repeat data ($n=341$) were recorded. This leads to an analytical sample size of 1887 men.

Hormone assays

T was measured by liquid chromatography–tandem mass spectrometry, with paired baseline and follow-up samples

analysed simultaneously (22). LH, FSH and SHBG were measured by the E170 platform electrochemiluminescence immunoassay (Roche Diagnostics) (23). E_2 was measured by both radioimmunoassay (at both phases) and mass spectrometry (at baseline). Free (F) T was calculated using the Vermeulen formula (24). Intra- and interassay coefficients of variation (CVs) were 4.0 and 5.6% for T; 1.7 and 3.2% for SHBG; 1.9 and 3.0% for LH; 1.8 and 5.3% for FSH; and 5.2 and 9.1% for E_2 (radioimmunoassay) and 3.5 and 3.7% for E_2 (GC-MS) respectively. The detection limit for the reproductive hormones were 0.55 nmol/L or 0.16 µg/L for total T (TT), 8.80 nmol/L or 10.00 µg/L for SHBG, 0.10 U/L for LH, 0.61 U/L for FSH, 18.14 pmol/L or 4.94 ng/L for radioimmunoassay E_2 and 9.91 pmol/L or 2.70 ng/L for GC-MS E_2 .

Other measures

Participants provided information on their self-rated general health (SF-36 questionnaire) and were asked whether they were currently being treated for the following medical conditions: heart problems, stroke, hypertension, diabetes, bronchitis, cancer, kidney or liver disease. The presence of heart problems, stroke or hypertension was

Figure 1

Flow chart.

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indicative of cardiovascular disease. The responses from the participants were further classified as either 'none' or 'one or more' or 'two or more' reported comorbidities from the eight chronic conditions. Self-reported poor health status was assessed using responses from participants on the SF-36 questionnaire concerning how the participants rated their overall general health. Self-reported poor health status was considered if responses included 'fair' or 'poor'.

Statistical analyses

The relationship between the AR CAG repeat and outcomes (ASEs) was assessed using the AR CAG repeat both as a continuous predictor and a tertiled categorical (tertile 1: 6–20 ($n=581$), tertile 2: 21–23 ($n=667$) and tertile 3: 24–39 ($n=639$) CAG repeats) predictor.

Outcomes such as changes in blood pressure, body composition, heel ultrasound, physical activity,

carbohydrate and lipid metabolism, cognitive processing speed (as measured via the DSST) and biochemical parameters, as well as the international prostate symptom score (IPSS), prostate-specific antigen (PSA) and reproductive hormone levels, were treated as continuous outcomes. In addition, in order to assess the relationship between the AR CAG repeat and changes in sexual, physical and psychological function, individual scores on the EMAS sexual function questionnaire, as well as the SF-36 and BDI, were used as continuous outcomes. Changes in ASEs were defined by the absolute change of an ASE (i.e. follow-up ASE value – baseline ASE value) adjusted for the baseline ASE value. The relationship between the AR CAG repeat (predictor) and the development of medical conditions or self-reported poor health status (outcome variables) was also assessed. The development of a medical condition was defined as subjects who reported being treated for a medical condition at follow up who did not report having the condition at baseline.

Table 1 Longitudinal changes in candidate androgen-sensitive parameters associated with the number of CAG repeats in the androgen receptor (linear regression). Data are expressed as standardized beta regression coefficients (95% CI).

Parameter (difference)	n	Model 1	Model 2	Model 3	Model 4
		Unadjusted	Adjusted for age and centre	Adjusted for age, centre and baseline total testosterone	Adjusted for age, centre and baseline oestradiol
Systolic blood pressure (mmHg)	1836	–0.02 (–0.06, 0.02)	–0.02 (–0.06, 0.02)	–0.02 (–0.06, 0.02)	–0.02 (–0.06, 0.02)
Diastolic blood pressure (mmHg)	1835	–0.03 (–0.07, 0.01)	–0.03 (–0.07, 0.01)	–0.03 (–0.07, 0.01)	–0.03 (–0.07, 0.01)
BMI (kg/m ²)	1812	0.00 (–0.04, 0.05)	0.00 (–0.04, 0.05)	0.00 (–0.04, 0.05)	0.01 (–0.04, 0.05)
Waist circumference (cm)	1830	–0.03 (–0.08, 0.02)	–0.03 (–0.08, 0.01)	–0.03 (–0.08, 0.01)	–0.03 (–0.07, 0.02)
MUAC (cm)	1827	–0.01 (–0.06, 0.03)	–0.01 (–0.06, 0.03)	–0.01 (–0.06, 0.03)	–0.02 (–0.06, 0.02)
PASE	1584	–0.00 (–0.05, 0.04)	–0.00 (–0.05, 0.04)	–0.00 (–0.05, 0.04)	–0.00 (–0.05, 0.04)
50 ft walk (s)	1807	0.04 (–0.01, 0.08)	0.04 (–0.01, 0.08)	0.04 (0.00, 0.09)*	0.04 (–0.00, 0.08)
PPT rating	1735	–0.01 (–0.06, 0.03)	–0.02 (–0.06, 0.02)	–0.02 (–0.06, 0.02)	–0.02 (–0.06, 0.02)
Fasting plasma glucose (mmol/L)	1772	0.02 (–0.02, 0.06)	0.02 (–0.02, 0.06)	0.02 (–0.02, 0.07)	0.02 (–0.02, 0.06)
HOMA-IR	1557	0.01 (–0.03, 0.04)	0.01 (–0.03, 0.04)	0.01 (–0.02, 0.05)	0.00 (–0.03, 0.04)
Total cholesterol (mmol/L)	1771	0.00 (–0.04, 0.04)	0.00 (–0.04, 0.04)	–0.00 (–0.04, 0.04)	0.01 (–0.04, 0.05)
HDL-cholesterol (mmol/L)	1763	–0.01 (–0.06, 0.03)	–0.02 (–0.06, 0.03)	–0.02 (–0.06, 0.02)	–0.02 (–0.06, 0.03)
LDL-cholesterol (mmol/L)	1696	0.02 (–0.02, 0.06)	0.02 (–0.02, 0.06)	0.02 (–0.02, 0.06)	0.02 (–0.02, 0.06)
Triglycerides (mmol/L)	1773	–0.03 (–0.08, 0.01)	–0.03 (–0.08, 0.01)	–0.03 (–0.07, 0.01)	–0.03 (–0.07, 0.02)
PSA (ng/mL)	1475	0.01 (–0.04, 0.06)	0.01 (–0.04, 0.06)	0.01 (–0.04, 0.06)	0.01 (–0.04, 0.06)
IPSS scores	1719	–0.04 (–0.09, 0.00)	–0.04 (–0.09, 0.00)	–0.04 (–0.09, 0.00)	–0.05 (–0.09, –0.00)*
Hb (g/L)	1539	0.00 (–0.04, 0.05)	0.00 (–0.04, 0.05)	–0.00 (–0.05, 0.05)	–0.01 (–0.05, 0.04)
DSST	1796	–0.02 (–0.07, 0.02)	–0.03 (–0.07, 0.02)	–0.03 (–0.07, 0.02)	–0.02 (–0.07, 0.02)
US-BMD (g/cm ²)	1773	–0.02 (–0.07, 0.02)	–0.02 (–0.07, 0.02)	–0.02 (–0.07, 0.02)	–0.02 (–0.07, 0.02)
US-BUA (dBIMHz/cm)	1742	0.02 (–0.02, 0.07)	0.02 (–0.02, 0.07)	0.03 (–0.02, 0.07)	0.03 (–0.02, 0.07)
US-SOS (kHz)	1739	0.02 (–0.03, 0.06)	0.02 (–0.03, 0.06)	0.02 (–0.02, 0.07)	0.02 (–0.02, 0.07)

BDI, Beck depression inventory score; BMD, bone mineral density; BMI, body mass index; BUA, broadband ultrasound attenuation; DSST, digital symbol substitution test; FPG, fasting plasma glucose concentration; Hb, plasma haemoglobin concentration; HDL-cholesterol, high-density lipid cholesterol; HOMA-IR, homeostatic model of insulin resistance; IPSS, international prostate symptom score; LDL-cholesterol, low-density lipid cholesterol; MUAC, mid upper arm circumference; PASE, physical activity scale for the elderly; PPT, physical performance test; PSA, serum prostate-specific antigen concentration; SF-36, medical outcome study short form 36-item questionnaire; SOS, speed of sound; WC, waist circumference.

* $P < 0.05$.

Table 2 Baseline and follow-up candidate ageing-related parameters of men with complete AR CAG repeat data. Data are expressed as unadjusted mean $\pm$ s.d. for continuous variables and as n (%) for categorical variables.

Parameter	Baseline ($n=1887$)	Follow up ($n=1887$)
Study age (years)	58.3 $\pm$ 10.5	62.7 $\pm$ 10.5***
BP (mmHg)		
Systolic	144.4 $\pm$ 19.8	146.2 $\pm$ 19.7***
Diastolic	87.0 $\pm$ 11.8	84.6 $\pm$ 11.6***
BMI (kg/m ²)	27.6 $\pm$ 3.9	27.8 $\pm$ 4.2***
WC (cm)	98.0 $\pm$ 10.6	99.5 $\pm$ 11.3***
MUAC (cm)	27.8 $\pm$ 2.6	27.2 $\pm$ 2.7***
PASE	205.0 $\pm$ 89.1	181.4 $\pm$ 96.0***
50 ft walk (s)	13.1 $\pm$ 2.5	14.0 $\pm$ 3.7***
PPT rating	24.3 $\pm$ 2.4	23.7 $\pm$ 2.5***
FPG (mmol/L)	5.6 $\pm$ 1.2	5.5 $\pm$ 1.3
HOMA-IR	3.1 $\pm$ 4.1	3.0 $\pm$ 2.9**
Chol (mmol/L)		
Total	5.6 $\pm$ 1.0	5.2 $\pm$ 1.1***
HDL	1.4 $\pm$ 0.4	1.4 $\pm$ 0.4***
LDL	3.5 $\pm$ 0.9	3.2 $\pm$ 1.0***
Triglycerides (mmol/L)	1.6 $\pm$ 1.2	1.5 $\pm$ 2.0
PSA (ng/mL)	1.6 $\pm$ 2.6	2.1 $\pm$ 6.5**
IPSS scores	5.2 $\pm$ 5.7	6.3 $\pm$ 6.2***
Hb (g/L)	150.3 $\pm$ 10.4	149.8 $\pm$ 11.6
DSST	28.9 $\pm$ 8.4	27.8 $\pm$ 9.0***
US-BMD (g/cm ²)	0.6 $\pm$ 0.1	0.9 $\pm$ 14.9
US-BUA (dBIMHz/cm)	81.0 $\pm$ 19.0	83.1 $\pm$ 18.2***
US-SOS (kHz)	1552.6 $\pm$ 34.2	1550.8 $\pm$ 32.9***
TS (nmol/L)	17.0 $\pm$ 5.9	16.6 $\pm$ 6.0***
F-TS (pmol/L)	305.1 $\pm$ 85.7	289.6 $\pm$ 86.7***
SHBG (nmol/L)	41.6 $\pm$ 18.4	44.1 $\pm$ 19.7***
LH (U/L)	5.8 $\pm$ 3.8	6.2 $\pm$ 4.5***
FSH (U/L)	7.8 $\pm$ 7.5	8.2 $\pm$ 8.6***
TT:LH ratio	3.6 $\pm$ 1.9	3.5 $\pm$ 2.0***
LH $\times$ TT product	101.2 $\pm$ 76.5	103.3 $\pm$ 76.9
E ₂ (pmol/L; RIA)	91.3 $\pm$ 27.9	90.6 $\pm$ 35.2
E ₂ (pmol/L; GC-MS)	73.6 $\pm$ 24.9	—
E ₂ :TT ratio	6.0 $\pm$ 4.1	6.1 $\pm$ 3.9
Overall sexual function	21.0 $\pm$ 6.5	21.1 $\pm$ 6.9***
SF-36 physical function	51.1 $\pm$ 7.5	50.5 $\pm$ 8.1**
BDI total	6.5 $\pm$ 6.1	6.2 $\pm$ 6.4*
SF-36 mental function	52.2 $\pm$ 8.7	52.1 $\pm$ 9.0
SF-36 vitality	15.2 $\pm$ 2.8	15.0 $\pm$ 2.9*
MEF score	3.5 $\pm$ 1.9	3.4 $\pm$ 1.9*
FOST score	5.2 $\pm$ 2.0	4.8 $\pm$ 2.1***
EF score	1.9 $\pm$ 1.0	2.1 $\pm$ 1.0***
VA score	2.2 $\pm$ 0.7	2.1 $\pm$ 0.8**
LW score	2.9 $\pm$ 0.4	2.8 $\pm$ 0.5***
UTB score	2.7 $\pm$ 0.6	2.6 $\pm$ 0.6
Sadness score	4.2 $\pm$ 0.9	4.3 $\pm$ 0.9
LOE score	0.6 $\pm$ 0.6	0.6 $\pm$ 0.6*
Fatigue score	0.5 $\pm$ 0.6	0.5 $\pm$ 0.6*
Poor health, n (%)	366 (19.6)	429 (23.7)***
≥ 1 illnesses, n (%)	763 (40.4)	1062 (56.3)***
≥ 2 illnesses, n (%)	290 (20.5)	586 (41.5)***

(Continued)

Table 2 Continued.

Parameter	Baseline ($n=1887$)	Follow up ($n=1887$)
Diabetes, n (%)	115 (6.2)	154 (8.4)***
CVD, n (%)	596 (32.0)	781 (44.2)***
Cancer, n (%)	85 (4.5)	148 (8.3)***
Prostate disease, n (%)	27 (8.0)	170 (9.3)

BDI, Beck depression inventory score; BMD, bone mineral density; BMI, body mass index; BUA, broadband ultrasound attenuation; CVD, cardiovascular disease; DSST, digital symbol substitution test; FPG, fasting plasma glucose concentration; Hb, plasma haemoglobin concentration; HOMA-IR, homeostatic model of insulin resistance; IPSS, international prostate symptom score; MUAC, mid upper arm circumference; PASE, physical activity scale for the elderly; PPT, physical performance test; PSA, serum prostate-specific antigen concentration; SF-36, medical outcome study short form 36-item questionnaire; SOS, speed of sound; WC, waist circumference; BP, blood pressure; Chol, cholesterol; TS, testosterone; F-TS, free testosterone; RIA, radioimmunoassay; MOF, morning erection frequency; FOST, frequency of sexual thoughts; EF, erectile frequency; VA, vigorous activity; LW, limited walking; UTB, unable to bend; LOE, loss of energy. Longitudinal (unadjusted) within-group differences: * $P < 0.05$ as assessed by paired t -test for continuous variables or McNemar's test for binary variables; ** $P < 0.01$ as assessed by paired t -test for continuous variables or McNemar's test for binary variables; *** $P < 0.001$ as assessed by paired t -test for continuous variables or McNemar's test for binary variables.

The development of self-reported poor health status was defined in a similar manner.

Linear regression was used to determine the longitudinal associations between the AR CAG repeat and each of the ASEs with results expressed as absolute differences (β -coefficients) and 95% confidence intervals (CI). Logistic regression analysis was used to assess the relationships between the AR CAG repeat and the development of medical conditions or self-reported poor health status (binary outcomes) with results expressed as odds ratios and 95% CI. For both linear and logistic regression analyses, adjustments were made for age, centre and baseline TT and (GC-MS) E₂ levels. The cut-off value for statistical significance was set to $P < 0.01$, when using the AR CAG repeat as a continuous linear predictor, in order to account for potential false-positive results, as used in our baseline cross-sectional analysis (18). In order to account for the multiple comparisons performed when using the AR CAG repeat as a tertiled predictor, a Bonferroni correction was applied, which lowered the threshold for statistical significance to $P < 0.003$. Statistical thresholds of $P < 0.05$, $P < 0.01$ and $P < 0.003$ are included in each of the tables, but only statistical thresholds of $P < 0.01$ (Tables 1, 3, 4, 5 and 6) and $P < 0.003$ (Supplementary Tables 1, 3, 4, 5, 6 and 7, see section on supplementary data given at the end of this article) are deemed significant. All statistical analyses were performed using STATA 13 SE (<http://www.stata.com>).

Results

Characteristics of the study subjects at baseline and follow up (including the distribution of the AR CAG repeat length)

Men with complete AR CAG repeat data were middle-aged, often overweight, with a relatively low prevalence of comorbidity burden, and with reproductive hormone levels within the eugonadal range. Most clinical endpoints changed over time in men with complete AR CAG repeat data except for glucose, triglycerides, haemoglobin, heel bone mineral density (US-BMD), androgen insensitivity index (LH×TT product), E₂ levels, aromatase activity (E₂:TT ratio), mental function (SF-36 mental function), inability to bend, sadness and prevalence of prostate disease in unadjusted analysis (Table 2). The distribution of the AR CAG repeat length approximated a normal distribution (data not shown) with mean ± S.D. = 22 ± 3 CAG repeats and a range of 6–39 CAG repeats. The distribution of the AR CAG repeat was similar in men with complete AR CAG repeat data, when compared with men who were excluded (Supplementary Table 2), indicating a low risk from selection bias.

Changes in body composition; heel ultrasound; and physical, prostate and cognitive function

The AR CAG repeat was not associated with changes in body composition parameters, such as BMI, waist circumference and mid-upper arm circumference (MUAC), and heel ultrasound parameters (US-BMD, US-BUA and US-SOS) (Table 2). The AR CAG repeat was not associated with changes in physical activity (PASE) or physical performance (50ft walk test and PPT rating) scores. The AR CAG repeat was not associated with changes in indices of prostate function, such as PSA levels or IPSS scores.

In addition, the AR CAG repeat was not associated with changes in cognitive processing speed, as assessed via DSST scores (Table 1). Adjustment for baseline TT or E₂ levels did not change the results obtained. Results were similar when using the AR CAG repeat as a tertiled categorical predictor (Supplementary Table 1). However, when using a less-stringent *P* value threshold, the AR CAG repeat was associated with changes in 50ft walking distance, limited walking, decreased vigorous activity and IPSS scores.

Changes in carbohydrate and lipid metabolism, blood pressure and haematological parameters

The AR CAG repeat was not associated with changes in fasting plasma glucose levels or a measure of insulin resistance (HOMA-IR). In addition, the AR CAG repeat was not associated with changes in total cholesterol, HDL-cholesterol, LDL-cholesterol or triglyceride levels.

The AR CAG repeat was not associated with changes in blood pressure or haemoglobin levels (Table 1). Adjustment for baseline TT or E₂ levels did not change the results obtained. Results were similar when using the AR CAG repeat as a tertiled categorical predictor (Supplementary Table 1). However, when using a less-stringent *P* value threshold, the AR CAG repeat was associated with changes in fasting glucose, HDL-cholesterol and triglyceride levels.

Changes in reproductive hormone levels and phase 2 reproductive hormone levels

The AR CAG repeat was not associated with changes in either TT or FT levels (Table 3). The AR CAG repeat was not associated with changes in LH, FSH, TT:LH ratio or the LH×TT product. However, the AR CAG repeat was positively associated with TT, FT and E₂ levels, but not

Table 3 Longitudinal changes in reproductive hormone levels associated with the number of CAG repeats in the androgen receptor (linear regression). Data are expressed as standardized beta regression coefficients (95% CI).

Parameter (difference)	n	Model 1	Model 2	Model 3	Model 4
		Unadjusted	Adjusted for age and centre	Adjusted for age, centre and baseline total testosterone	Adjusted for age, centre and baseline oestradiol
Total testosterone (nmol/L)	1887	0.02 (−0.02, 0.07)	0.02 (−0.02, 0.07)	–	0.02 (−0.02, 0.07)
Free testosterone (pmol/L)	1866	0.03 (−0.01, 0.07)	0.03 (−0.01, 0.07)	–	0.03 (−0.01, 0.07)
SHBG (nmol/L)	1866	0.01 (−0.03, 0.06)	0.01 (−0.03, 0.06)	0.01 (−0.04, 0.05)	0.01 (−0.03, 0.06)
LH (U/L)	1864	−0.02 (−0.07, 0.02)	−0.02 (−0.06, 0.03)	−0.02 (−0.06, 0.03)	−0.02 (−0.07, 0.02)
FSH (U/L)	1865	−0.02 (−0.06, 0.03)	−0.02 (−0.06, 0.03)	−0.02 (−0.06, 0.03)	−0.02 (−0.07, 0.02)
E ₂ (pmol/L; radioimmunoassay)	1859	0.02 (−0.02, 0.06)	0.02 (−0.02, 0.07)	0.02 (−0.03, 0.06)	–
E ₂ :TT ratio	1859	0.02 (−0.02, 0.06)	0.02 (−0.02, 0.06)	0.02 (−0.02, 0.06)	0.02 (−0.03, 0.06)
TT:LH ratio	1864	0.00 (−0.04, 0.05)	0.01 (−0.04, 0.05)	0.00 (−0.04, 0.04)	0.01 (−0.03, 0.05)
LH×TT product	1864	0.01 (−0.03, 0.05)	0.01 (−0.03, 0.05)	0.01 (−0.03, 0.05)	0.01 (−0.04, 0.05)

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Table 4 Reproductive hormone levels at follow up associated with the number of CAG repeats in the androgen receptor (linear regression). Data are expressed as standardized beta regression coefficients (95% CI).

Parameter (Phase 2)	n	Model 1	Model 2	Model 3	Model 4
		Unadjusted	Adjusted for age and centre	Adjusted for age, centre and baseline total testosterone	Adjusted for age, centre and baseline oestradiol
Total testosterone (nmol/L)	1887	0.07 (0.02, 0.11) [‡]	0.07 (0.02, 0.11) [‡]	–	0.04 (–0.00, 0.08)
Free testosterone (pmol/L)	1871	0.08 (0.04, 0.13) [§]	0.08 (–0.04, 0.12) [§]	–	0.06 (0.02, 0.10) [‡]
SHBG (nmol/L)	1871	0.00 (–0.04, 0.05)	0.01 (–0.03, 0.05)	–0.03 (–0.06, 0.00)	–0.01 (–0.05, 0.03)
LH (U/L)	1871	–0.01 (–0.06, 0.03)	–0.01 (–0.05, 0.03)	–0.01 (–0.06, 0.03)	–0.02 (–0.06, 0.03)
FSH (U/L)	1871	–0.04 (–0.08, 0.01)	–0.03 (–0.08, 0.01)	–0.03 (–0.07, 0.01)	–0.03 (–0.08, 0.01)
E ₂ (pmol/L; radioimmunoassay)	1865	0.07 (0.03, 0.12) [‡]	0.07 (0.03, 0.12) [‡]	0.06 (0.01, 0.10) [*]	–
E ₂ :TT ratio	1865	0.02 (–0.03, 0.06)	0.02 (–0.03, 0.06)	0.04 (–0.00, 0.08)	0.01 (–0.03, 0.06)
TT:LH ratio	1871	0.04 (–0.00, 0.09)	0.04 (–0.00, 0.08)	0.02 (–0.02, 0.06)	0.03 (–0.01, 0.08)
LH×TT product	1871	0.03 (–0.02, 0.07)	0.03 (–0.01, 0.07)	0.00 (–0.04, 0.04)	0.01 (–0.03, 0.05)

* $P < 0.05$; [‡] $P < 0.01$; [§] $P < 0.001$.

the E₂:TT ratio, at follow up, in a cross-sectional manner (Table 4). After adjustment for baseline E₂ levels, the cross-sectional relationship between the AR CAG and TT levels became non-significant. Results were similar when using the AR CAG repeat as a tertiled categorical predictor (Supplementary Table 3). However, when using a less-stringent *P*-value threshold, the AR CAG repeat was associated with changes in TT, FT, E₂ and LH levels and the LH×TT product.

Changes in sexual, physical, psychological, mental and quality-of-life questionnaire scores

The AR CAG repeat was not associated with changes in sexual, physical or psychological function questionnaire scores. In addition, the AR CAG repeat was not associated

with changes in overall sexual function (SFQ-OSF), overall physical function (SF-36 physical function), psychological (BDI total) and mental function (SF-36 mental function), and quality-of-life (SF-36 vitality) scores (Table 5). Adjustment for baseline TT or E₂ levels did not change the results obtained. Results were similar when using the AR CAG repeat as a tertiled categorical predictor (Supplementary Tables 4, 5 and 6). However, when using a less-stringent *P* value threshold, the AR CAG repeat was associated with changes in overall sexual function, fatigue, mental function and vitality scores.

Changes in medical conditions

The AR CAG repeat was not associated with the development of self-reported poor health status, comorbidity or multi-

Table 5 Longitudinal changes in sexual, physical, psychological, mental and quality of life questionnaire scores associated with the number of CAG repeats in the androgen receptor (AR) (linear regression). Data are expressed as standardized beta regression coefficients (95% CI).

Parameter (difference)	n	Model 1	Model 2	Model 3	Model 4
		Unadjusted	Adjusted for age and centre	Adjusted for age, centre and baseline total testosterone	Adjusted for age, centre and baseline oestradiol
Morning erection scores	1704	–0.02 (–0.07, 0.02)	–0.02 (–0.06, 0.02)	–0.02 (–0.06, 0.02)	–0.02 (–0.06, 0.02)
Sexual thoughts scores	1715	–0.02 (–0.06, 0.02)	–0.02 (–0.06, 0.02)	–0.02 (–0.06, 0.02)	–0.02 (–0.06, 0.02)
Erectile function scores	1652	–0.00 (–0.05, 0.04)	–0.00 (–0.05, 0.04)	–0.00 (–0.05, 0.04)	–0.01 (–0.05, 0.04)
SFQ-OSF scores	1167	–0.02 (–0.08, 0.03)	–0.01 (–0.07, 0.04)	–0.02 (–0.07, 0.03)	–0.01 (–0.07, 0.04)
Vigorous activity score	1797	–0.00 (–0.04, 0.04)	–0.00 (–0.04, 0.04)	–0.00 (–0.04, 0.04)	–0.00 (–0.04, 0.04)
Limited walking score	1781	–0.02 (–0.07, 0.02)	–0.02 (–0.06, 0.02)	–0.02 (–0.07, 0.02)	–0.02 (–0.06, 0.02)
Unable to bend	1794	–0.02 (–0.06, 0.02)	–0.01 (–0.05, 0.02)	–0.01 (–0.05, 0.03)	–0.01 (–0.05, 0.03)
SF-36 physical function	1721	–0.01 (–0.05, 0.03)	–0.01 (–0.05, 0.03)	–0.01 (–0.05, 0.03)	–0.01 (–0.05, 0.03)
Sadness score	1773	0.02 (–0.02, 0.06)	0.02 (–0.02, 0.06)	0.02 (–0.02, 0.06)	0.02 (–0.02, 0.06)
Loss of energy score	1814	–0.01 (–0.05, 0.03)	–0.01 (–0.05, 0.03)	–0.01 (–0.05, 0.03)	–0.02 (–0.06, 0.02)
Fatigue score	1816	–0.01 (–0.05, 0.03)	–0.01 (–0.05, 0.03)	–0.01 (–0.05, 0.03)	–0.01 (–0.05, 0.03)
BDI total	1793	–0.03 (–0.07, 0.01)	–0.03 (–0.07, 0.01)	–0.03 (–0.07, 0.01)	–0.03 (–0.07, 0.01)
SF-36 mental function	1720	0.03 (–0.01, 0.08)	0.04 (–0.01, 0.08)	0.04 (–0.01, 0.08)	0.04 (–0.01, 0.08)
SF-36 vitality	1792	0.02 (–0.03, 0.06)	0.02 (–0.03, 0.06)	0.01 (–0.03, 0.06)	0.02 (–0.02, 0.06)

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Table 6 Development of medical conditions associated with the number of CAG repeats in the androgen receptor (logistic regression). Data are expressed as odds ratios (95% CI).

Parameter (development)	n	Model 1	Model 2	Model 3	Model 4
		Unadjusted	Adjusted for age and centre	Adjusted for age, centre and baseline total testosterone	Adjusted for age, centre and baseline oestradiol
Poor health	1445	1.05 (1.00, 1.11)*	1.05 (1.00, 1.11)*	1.06 (1.00, 1.11)*	1.05 (1.00, 1.11)*
≥1 illness	1124	0.99 (0.95, 1.03)	0.99 (0.95, 1.04)	1.00 (0.95, 1.04)	1.00 (0.96, 1.04)
≥2 illness	939	1.00 (0.95, 1.06)	1.00 (0.95, 1.06)	1.01 (0.95, 1.06)	1.01 (0.96, 1.07)
Diabetes	1687	0.97 (0.89, 1.06)	0.97 (0.89, 1.06)	0.99 (0.90, 1.08)	0.98 (0.89, 1.07)
Cardiovascular disease	1172	1.01 (0.96, 1.06)	1.01 (0.97, 1.06)	1.02 (0.97, 1.07)	1.01 (0.97, 1.06)
Cancer	1703	1.04 (0.97, 1.13)	1.05 (0.97, 1.13)	1.04 (0.97, 1.12)	1.05 (0.97, 1.13)
Prostate disease	1646	1.01 (0.95, 1.08)	1.01 (0.95, 1.08)	1.02 (0.95, 1.09)	1.02 (0.95, 1.09)

* $P < 0.05$.

morbidity burden or any other medical conditions (Table 6). Adjustment for baseline TT or E_2 levels did not change the results obtained. Results were similar when using the AR CAG repeat as a tertiled categorical predictor (Supplementary Table 7). However, when using a less-stringent P value threshold, the AR CAG repeat was associated with the development of poor health.

Discussion

The main finding from this longitudinal study was the lack of association between the AR CAG repeat and changes in a wide variety of putative ASEs and medical conditions potentially important in the phenotype of ageing in men. AR CAG repeat lengths, treated as a continuous variable (Tables 1, 3, 5 and 6) or separated into tertiles (Supplementary Tables 1, 3, 4, 5, 6 and 7), showed similar results.

Our findings differed from those presented by Krithivas and colleagues (9). They reported that the AR CAG repeat was associated with the magnitude of the longitudinal decline in T levels within the MMAS, which we did not observe in our study. However, their study had a longer follow-up period (approximately 8 vs 4 years) than EMAS, but contained a smaller sample size than EMAS ($n = 1709$ men vs $n = 3369$ men). Their study used the radioimmunoassay to measure T, which is known to have a suboptimal performance at low levels (11, 25). In their study, the relationship between reproductive hormone levels and the AR CAG repeat was investigated per three AR CAG repeats, which may not represent a clinically meaningful increase. Finally, in their study, quantification of the decline in T levels over time in relation to the AR CAG repeat length was performed on pairing of just four individuals based on identical baseline TT, age and waist-to-hip ratio.

Our findings agree with those from Travison and colleagues (26), which indicate that the AR CAG repeat is not associated with changes in reproductive hormone levels within the MMAS. The study by Travison *et al.* (26) used similar methodology as in Krithivas *et al.* (9) and may suffer from similar limitations. However, Travison and colleagues studied the change in reproductive hormone levels using the AR CAG repeat length as a continuous measure. Our study investigated the change in a large number of endpoints in relation to the AR CAG repeat, assessed as either a continuous or tertiled predictor, and might be more similar to the study by Travison and colleagues.

Zitzmann and colleagues have proposed that the AR CAG repeat might be a putative biomarker for 'androgenicity' (1, 2, 10). Our results in men from the general population do not support this concept. Our cohort consisted of community-dwelling middle-aged and elderly European men from the general population, and the few subjects with diagnosed pituitary, testicular or adrenal disease were excluded from the analysis. Men in this study presumably have an intact HPT axis, and thus the potential consequences of any variations in the AR CAG repeat length are likely to be minimized or rendered clinically insignificant by compensatory regulatory feedback changes involving gonadotrophins and E_2 , although no relationship between the AR CAG repeat length and longitudinal changes in either could be observed. Our findings did not exclude the possibility that in men who have either pituitary or testicular deficits, in whom the feedback regulation has been disrupted, the AR CAG repeat may have an impact on the severity of symptoms associated with androgen deficiency or the response to T replacement therapy.

The present cross-sectional results at follow up confirmed our earlier finding at baseline (18) that longer AR CAG repeat length was associated with higher E_2

levels. However, we did not observe that the AR CAG repeat length was associated with longitudinal changes in E_2 levels. Our longitudinal results indicate that variations in AR CAG repeat length may not contribute to the phenotype of ageing, over and above, which could be associated with the age-related decline in T levels. Our findings have to be interpreted with caution, as a large number of endpoints were assessed in relation to a single genetic marker. The relationship between the AR CAG repeat and changes in ASEs was unclear before this study. Although we have reported all results, we are cautious in interpreting associations, which are above our P value thresholds ($P > 0.01$ and $P > 0.003$). We have chosen a more stringent P value threshold in line with recommendations proposed to account for multiplicity (27). However, our findings do suggest that the effect of the AR CAG repeat on changes in phenotypic endpoints in ageing men is small.

Strengths and limitations

EMAS is a multi-centre European longitudinal cohort study that investigates the endocrine and metabolic determinants of male ageing, such as alterations in androgenic and anabolic hormone levels. The present analysis examined the temporal associations of within-subject differences in ASEs and medical conditions in relation to variations in a genetic marker of androgen action, which should not be influenced by reverse causality, as the AR CAG repeat length is fixed throughout life.

Both baseline and follow-up T levels from EMAS men were measured via liquid chromatography–tandem mass spectrometry, which minimized any potential method-related variation (25). The EMAS questionnaires related to sexual, physical and psychological function were carefully standardized and translated into local languages in eight centres (21, 28, 29, 30, 31, 32). Finally, men with missing AR CAG repeat data showed minor differences in baseline FT and follow-up FT, follow-up insulin resistance and follow-up vigorous activity scores, compared with the analytical sample after age and centre adjustment (Supplementary Table 8). Thus, potential bias due to missing AR CAG repeat data is likely to be minimal.

However, measurement of E_2 levels by the radioimmunoassay should be considered a limitation, due to its suboptimal performance at low hormone concentrations (33). Another limitation of this study is that it contains only middle-aged and elderly men of European origin. Thus, the

findings might not extend to younger men or individuals of a non-European background, as the AR CAG repeat length is known to differ across ethnic groups (34, 35). The median duration to follow up was 4.3 years, which may be too short to discern slower longitudinal changes associated with variation in the AR CAG repeat length.

Conclusions

We demonstrate in community-dwelling middle-aged and elderly men of European origin that variations in AR CAG repeat length are not longitudinally associated with short-term changes in ASEs or the development of medical conditions. The AR CAG repeat as a genetic marker of androgen action is unlikely to contribute to major changes in the phenotype of ageing men.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/EJE-16-0447>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

The European Male Ageing Study (EMAS) is funded by the Commission of the European Communities Fifth Framework Program 'Quality of Life and Management of Living Resources' Grant QLK6-CT-2001-00258 and facilitated by the Manchester Biomedical Research Centre and the NIHR Greater Manchester: Clinical Research Network. Additional support was also provided by Arthritis Research UK Centre for Epidemiology and the National Institute for Health Research and the Manchester Biomedical Research Centre. The principal investigator of EMAS is Professor Frederick Wu, M.D., Andrology Research Unit, University of Manchester, Manchester, UK.

Author contribution statement

R J A H E and F C W W wrote the analysis plan for the study. B G K provided lab measurements required to perform the analysis. R J A H E performed the analysis. I T H, S R P, T A, R D A, A G, N P, M K R, G T, R G and F C W W supervised the analysis. R J A H E wrote the paper. I T H, S R P, T A, T W O, G B, F F C, M M, R D A, A G, T S H, K K, M E J, M P, N P, B G K, D V, M K R, G T, R G and F C W W supervised the writing process.

Acknowledgements

R J A H E is grateful for the support received from the Biotechnology and Biological Sciences Research Council Doctoral Training Partnership (BBSRC-DTP), as well as the Fundatie van de Vrijvrouwe van Renswoude and Scholten-Cordes scholarship foundations. The authors thank the men who participated in the eight countries and the research/nursing staff in the eight centres: C Pott (Manchester), E Wouters (Leuven), M Nilsson

(Malmö), M del Mar Fernandez (Santiago de Compostela), M Jedrzejowska (Łódź), H-M Tabo (Tartu), A Heredi (Szeged) for their data collection and C Moseley (Manchester) for data entry and project coordination.

References

- 1 Zitzmann M. Mechanisms of disease: pharmacogenetics of testosterone therapy in hypogonadal men. *Nature Clinical Practice Urology* 2007 **4** 161–166. (doi:10.1038/ncpuro0706)
- 2 Zitzmann M. The role of the CAG repeat androgen receptor polymorphism in andrology. *Advances in the Management of Testosterone Deficiency* 2009 **37** 52–61. (doi:10.1159/000175843)
- 3 Gottlieb B, Trifiro M, Lumbroso R & Pinsky L. The androgen receptor gene mutations database. *Nucleic Acids Research* 1997 **25** 158–162. (doi:10.1093/nar/25.1.158)
- 4 La Spada AR, Wilson EM, Lubahn DB, Harding AE & Fischback KH. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature* 1991 **352** 77–79. (doi:10.1038/352077a0)
- 5 Zitzmann M, Gromoll J, von Eckardstein A & Nieschlag E. The CAG repeat polymorphism in the androgen receptor gene modulates body fat mass and serum concentrations of leptin and insulin in men. *Diabetologia* 2003 **46** 31–39. (doi:10.1007/s00125-002-0980-9)
- 6 Zitzmann M, Brune M, Kornmann B, Gromoll J, von Eckardstein S, von Eckardstein A & Nieschlag E. The CAG repeat polymorphism in the AR gene affects high density lipoprotein cholesterol and arterial vasoreactivity. *Journal of Clinical Endocrinology & Metabolism* 2001 **86** 4867–4873. (doi:10.1210/jc.86.10.4867)
- 7 Zitzmann M, Brune M, Kornmann B, Gromoll J, Junker R & Nieschlag E. The CAG repeat polymorphism in the androgen receptor gene affects bone density and bone metabolism in healthy males. *Clinical Endocrinology* 2001 **55** 649–657. (doi:10.1046/j.1365-2265.2001.01391.x)
- 8 Zitzmann M & Nieschlag E. Androgen receptor gene CAG repeat length and body mass index modulate the safety of long-term intramuscular testosterone undecanoate therapy in hypogonadal men. *Journal of Clinical Endocrinology & Metabolism* 2007 **92** 3844–3853. (doi:10.1210/jc.2007-0620)
- 9 Krithivas K, Yurgalevitch SM, Mohr BA, Wilcox CJ, Batter SJ, Brown M, Longcope C, McKinlay JB & Kantoff PW. Evidence that the CAG repeat in the androgen receptor gene is associated with the age-related decline in serum androgen levels in men. *Journal of Endocrinology* 1999 **162** 137–142. (doi:10.1677/joe.0.1620137)
- 10 Zitzmann M & Nieschlag E. The CAG repeat polymorphism within the androgen receptor gene and maleness. *International Journal of Andrology* 2003 **26** 76–83. (doi:10.1046/j.1365-2605.2003.00393.x)
- 11 Hsing AW, Stanczyk FZ, Belanger A, Schroeder P, Chang L, Falk RT & Fears TR. Reproducibility of serum sex steroid assays in men by RIA and mass spectrometry. *Cancer Epidemiology Biomarkers & Prevention* 2007 **16** 1004–1008. (doi:10.1158/1055-9965.epi-06-0792)
- 12 Hakimi JM, Schoenberg MP, Rondinelli RH, Piantadosi S & Barrack ER. Androgen receptor variants with short glutamine or glycine repeats may identify unique subpopulations of men with prostate cancer. *Clinical Cancer Research* 1997 **3** 1599–1608.
- 13 Ingles SA, Ross RK, Yu MC, Irvine RA, LaPera G, Haile RW & Coetzee GA. Association of prostate cancer risk with genetic polymorphisms in vitamin D receptor and androgen receptor. *Journal of the National Cancer Institute* 1997 **89** 166–170. (doi:10.1093/jnci/89.2.166)
- 14 Heinlein CA & Chang C. Androgen receptor in prostate cancer. *Endocrine Reviews* 2004 **25** 276–308. (doi:10.1210/er.2002-0032)
- 15 Nenonen HA, Giwercman A, Hallengren E & Giwercman YL. Non-linear association between androgen receptor CAG repeat length and risk of male subfertility – a meta-analysis. *International Journal of Andrology* 2011 **34** 327–332. (doi:10.1111/j.1365-2605.2010.01084.x)
- 16 Van Pottelbergh I, Lumbroso S, Goemaere S, Sultan C & Kaufman JM. Lack of influence of the androgen receptor gene CAG-repeat polymorphism on sex steroid status and bone metabolism in elderly men. *Clinical Endocrinology* 2001 **55** 659–666. (doi:10.1046/j.1365-2265.2001.01403.x)
- 17 Holgersson MB, Giwercman A, Bjartell A, Wu FCW, Huhtaniemi IT, O'Neill TW, Pendleton N, Vanderschueren D, Lean MEJ, Han TS *et al.* Androgen receptor polymorphism-dependent variation in prostate-specific antigen concentrations of European men. *Cancer Epidemiology Biomarkers & Prevention* 2014 **23** 2048–2056. (doi:10.1158/1055-9965.epi-14-0376)
- 18 Huhtaniemi IT, Pye SR, Limer KL, Thomson W, O'Neill TW, Platt H, Payne D, John SL, Jiang M, Boonen S *et al.* Increased estrogen rather than decreased androgen action is associated with longer androgen receptor CAG repeats. *Journal of Clinical Endocrinology & Metabolism* 2009 **94** 277–284. (doi:10.1210/jc.2008-0848)
- 19 Lee DM, O'Neill TW, Pye SR, Silman AJ, Finn JD, Pendleton N, Tajar A, Bartfai G, Casanueva F, Forti G *et al.* The European Male Ageing Study (EMAS): design, methods and recruitment. *International Journal of Andrology* 2009 **32** 11–24. (doi:10.1111/j.1365-2605.2008.00879.x)
- 20 Lee DM, Pye SR, Tajar A, O'Neill TW, Finn JD, Boonen S, Bartfai G, Casanueva FF, Forti G, Giwercman A *et al.* Cohort profile: the European Male Ageing Study. *International Journal of Epidemiology* 2013 **42** 391–401. (doi:10.1093/ije/dyr234)
- 21 O'Connor DB, Corona G, Forti G, Tajar A, Lee DM, Finn JD, Bartfai G, Boonen S, Casanueva FF, Giwercman A *et al.* Assessment of sexual health in aging men in Europe: development and validation of the European Male Ageing Study sexual function questionnaire. *Journal of Sexual Medicine* 2008 **5** 1374–1385. (doi:10.1111/j.1743-6109.2008.00781.x)
- 22 Gallagher LM, Owen LJ & Keevil BG. Simultaneous determination of androstenedione and testosterone in human serum by liquid chromatography-tandem mass spectrometry. *Annals of Clinical Biochemistry* 2007 **44** 48–56. (doi:10.1258/00045630779595922)
- 23 Wu FC, Tajar A, Pye SR, Silman AJ, Finn JD, O'Neill TW, Bartfai G, Casanueva F, Forti G, Giwercman A *et al.* Hypothalamic-pituitary-testicular axis disruptions in older men are differentially linked to age and modifiable risk factors: the European Male Ageing Study. *Journal of Clinical Endocrinology & Metabolism* 2008 **93** 2737–2745. (doi:10.1210/jc.2007-1972)
- 24 Vermeulen A, Verdonck L & Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *Journal of Clinical Endocrinology & Metabolism* 1999 **84** 3666–3672. (doi:10.1210/jc.84.10.3666)
- 25 Huhtaniemi IT, Tajar A, Lee DM, O'Neill TW, Finn JD, Bartfai G, Boonen S, Casanueva FF, Giwercman A, Han TS *et al.* Comparison of serum testosterone and estradiol measurements in 3174 European men using platform immunoassay and mass spectrometry; relevance for the diagnostics in aging men. *European Journal of Endocrinology* 2012 **166** 983–991. (doi:10.1530/EJE-11-1051)
- 26 Travison TG, Shackelton R, Araujo AB, Morley JE, Williams RE, Clark RV & McKinlay JB. Frailty, serum androgens, and the CAG repeat polymorphism: results from the Massachusetts Male Aging Study. *Journal of Clinical Endocrinology & Metabolism* 2010 **95** 2746–2754. (doi:10.1210/jc.2009-0919)
- 27 Streiner DL & Norman GR. Correction for multiple testing is there a resolution? *Chest* 2011 **140** 16–18. (doi:10.1378/chest.11-0523)
- 28 Corona G, Lee DM, Forti G, O'Connor DB, Maggi M, O'Neill TW, Pendleton N, Bartfai G, Boonen S, Casanueva FF *et al.* Age-related changes in general and sexual health in middle-aged and older men: results from the European Male Ageing Study (EMAS). *Journal of Sexual Medicine* 2010 **7** 1362–1380. (doi:10.1111/j.1743-6109.2009.01601.x)
- 29 Tajar A, Forti G, O'Neill TW, Lee DM, Silman AJ, Finn JD, Bartfai G, Boonen S, Casanueva FF, Giwercman A *et al.* Characteristics of secondary, primary, and compensated hypogonadism in aging men:

- evidence from the European Male Ageing Study. *Journal of Clinical Endocrinology & Metabolism* 2010 **95** 1810–1818. (doi:10.1210/jc.2009-1796)
- 30 Tajar A, Huhtaniemi IT, O'Neill TW, Finn JD, Pye SR, Lee DM, Bartfai G, Boonen S, Casanueva FF, Forti G *et al.* Characteristics of androgen deficiency in late-onset hypogonadism: results from the European Male Aging Study (EMAS). *Journal of Clinical Endocrinology & Metabolism* 2012 **97** 1508–1516. (doi:10.1210/jc.2011-2513)
- 31 Wu FC, Tajar A, Beynon JM, Pye SR, Silman AJ, Finn JD, O'Neill TW, Bartfai G, Casanueva FF, Forti G *et al.* Identification of late-onset hypogonadism in middle-aged and elderly men. *New England Journal of Medicine* 2010 **363** 123–135. (doi:10.1056/NEJMoa0911101)
- 32 Lee DM, Tajar A, O'Neill TW, O'Connor DB, Bartfai G, Boonen S, Bouillon R, Casanueva FF, Finn JD, Forti G *et al.* Lower vitamin D levels are associated with depression among community-dwelling European men. *Journal of Psychopharmacology* 2011 **25** 1320–1328. (doi:10.1177/0269881110379287)
- 33 Taylor AE, Keevil B & Huhtaniemi IT. Mass spectrometry and immunoassay: how to measure steroid hormones today and tomorrow. *European Journal of Endocrinology* 2015 **173** D1–D12. (doi:10.1530/EJE-15-0338)
- 34 Ackerman CM, Lowe LP, Lee H, Hayes MG, Dyer AR, Metzger BE, Lowe WL & Urbanek M. Ethnic variation in allele distribution of the androgen receptor (AR) (CAG)(n) repeat. *Journal of Andrology* 2012 **33** 210–215. (doi:10.2164/jandrol.111.013391)
- 35 Nelson KA & Witte JS. Androgen receptor CAG repeats and prostate cancer. *American Journal of Epidemiology* 2002 **155** 883–890. (doi:10.1093/aje/155.10.883)

Received 25 May 2016

Revised version received 17 August 2016

Accepted 15 September 2016