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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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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 which are influenced by testosterone (T) levels in middle-aged and elderly European men.

Design: Multinational European observational prospective cohort study

Participants: 1887 men (mean±sd age: 63±11 years; median follow-up: 4.3 years) from centres of 8 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 both as a continuous and 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 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 timeframe, variations in the *AR* CAG repeat do not contribute to the rate of phenotypic ageing, over and above, that, which might be associated with the age-related decline in T levels.

Introduction

The length of the *Androgen Receptor (AR)* tri-nucleotide CAG repeat in exon 1, encoding a poly-glutamine 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 (1-4) 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, 11-14). 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 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 (LHxTT 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 the current study was to assess whether the *AR* CAG repeat length was 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 European Male Ageing Study (EMAS), as described elsewhere (19-21), 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 to 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, hematological, 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 the baseline assessment.

Exclusion criteria

As depicted in the flow chart (figure 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) was recorded. This lead 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). E2 was measured by both radio-immunoassay (at both phases) and by mass spectrometry (at baseline). Free (F) T was calculated using the Vermeulen formula (24). Intra- and interassay coefficients of variation (CVs) were: T, 4.0% and 5.6%; SHBG, 1.7% and 3.2%; LH, 1.9% and 3.0%; FSH, 1.8% and 5.3%, and (radio-immunoassay) E2 5.2% and 9.1% and (GC-MS) E2 3.5% and 3.7%, respectively. The detection limit for the reproductive hormones were: total T (TT) [0.55 nmol/L or 0.16 µg/L], SHBG [8.80 nmol/L or 10.00 µg/L], LH [0.10 U/L], FSH [0.61 U/L], radio-immunoassay E2 [18.14 pmol/L or 4.94 ng/L] and GC-MS E2 [9.91 pmol/L or 2.70 ng/L].

Other measures

Participants provided information on their self-rated general health (SF36 questionnaire) and were asked if 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 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 SF36 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, as well as a tertiled categorical [tertile1: 6-20 (n=581), tertile2: 21-23 (n=667) and tertile3: 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 SF36 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. 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 2a-2e) and $p<0.003$ (S2a-S2f) 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, hemoglobin, heel bone mineral density (US-BMD), androgen insensitivity index (LHxTT product), E2 levels, aromatase activity (E2:TT ratio), mental function (SF36 mental function), inability to bend, sadness, and prevalence of prostate disease in unadjusted analysis (Table 1). The distribution of the *AR* CAG repeat length approximated a normal distribution (data not shown) with mean±SD = 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 to men who were excluded (S1) indicating a low risk from selection bias.

Changes in body composition, heel ultrasound, 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, US-SOS) (Table 1a). The *AR* CAG repeat was not associated with changes in physical activity (PASE) or physical performance (50 feet 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 2a). 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 (S2a). 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 hematological 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 hemoglobin levels (Table 2a). 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 (S2a). 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 2b). The *AR* CAG repeat was not associated with changes in LH, FSH, TT:LH ratio or the LHxTT product. However, the *AR* CAG repeat was positively associated with TT, FT and E2 levels, but not the E2:TT ratio, at follow-up, in a cross-sectional manner (Table 2c). 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 (S2b). However, when using a less stringent p-value threshold, the *AR* CAG repeat was associated with changes in TT, FT, E2, LH levels and the LHxTT 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 (SF36 physical function), psychological (BDI-total) and mental function (SF36 mental function), and quality of life (SF36 vitality) scores (Table 2c). 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 (S2c, S2d and S2e). 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-morbidity burden, or any other medical conditions (Table 2e). 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 (S2f). 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 2a-2e) or separated into tertiles (S2a-2f), 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 radio-immunoassay to measure T, which is known to have a sub-optimal performance at low levels (11, 25). In their study, the relationship between reproductive hormone levels and the *AR* CAG repeat was investigated per 3 *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 4 individuals based on identical baseline TT, age and waist to hip ratio.

Our findings agree with those from Travison and colleagues (26), whom 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 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 the present 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 gonadotropins and E2, 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 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 E2 levels. However, we did not observe that the *AR* CAG repeat length was associated with longitudinal changes in E2 levels. Our longitudinal results indicate that variations in *AR* CAG repeat length may not contribute to the phenotype of ageing, over and above, that, which could be associated with the age-related decline in T levels. Our findings have to be interpreted with caution, since 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 prior to 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, which 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, since 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 8 centres (21, 28-32). 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, as compared to the analytical sample after age and centre adjustment (S2g). Thus, potential bias due to missing *AR* CAG repeat data is likely to be minimal.

However, measurement of E2 levels by the radio-immunoassay should be considered a limitation, due to its suboptimal performance at low hormone concentrations (33). Another limitation of the current 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, since 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 associated longitudinally 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.

Declaration of interest

The authors declare no conflicts of interest.

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Author contributions

RJAHE and FCWW wrote the analysis plan for the study. BGK provided lab measurements required to perform the analysis. RJAHE performed the analysis. ITH, SRP, TA, RDA, AG, NP, MKR, GT, RG and FCWW supervised the analysis. RJAHE wrote the paper. ITH, SRP, TA, TWO, GB, FFC, MM, RDA, AG, TSH, KK, MEJ, MP, NP, BGK, DV, MKR, GT, RG and FCWW supervised the writing process.

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Figure 1, flow chart

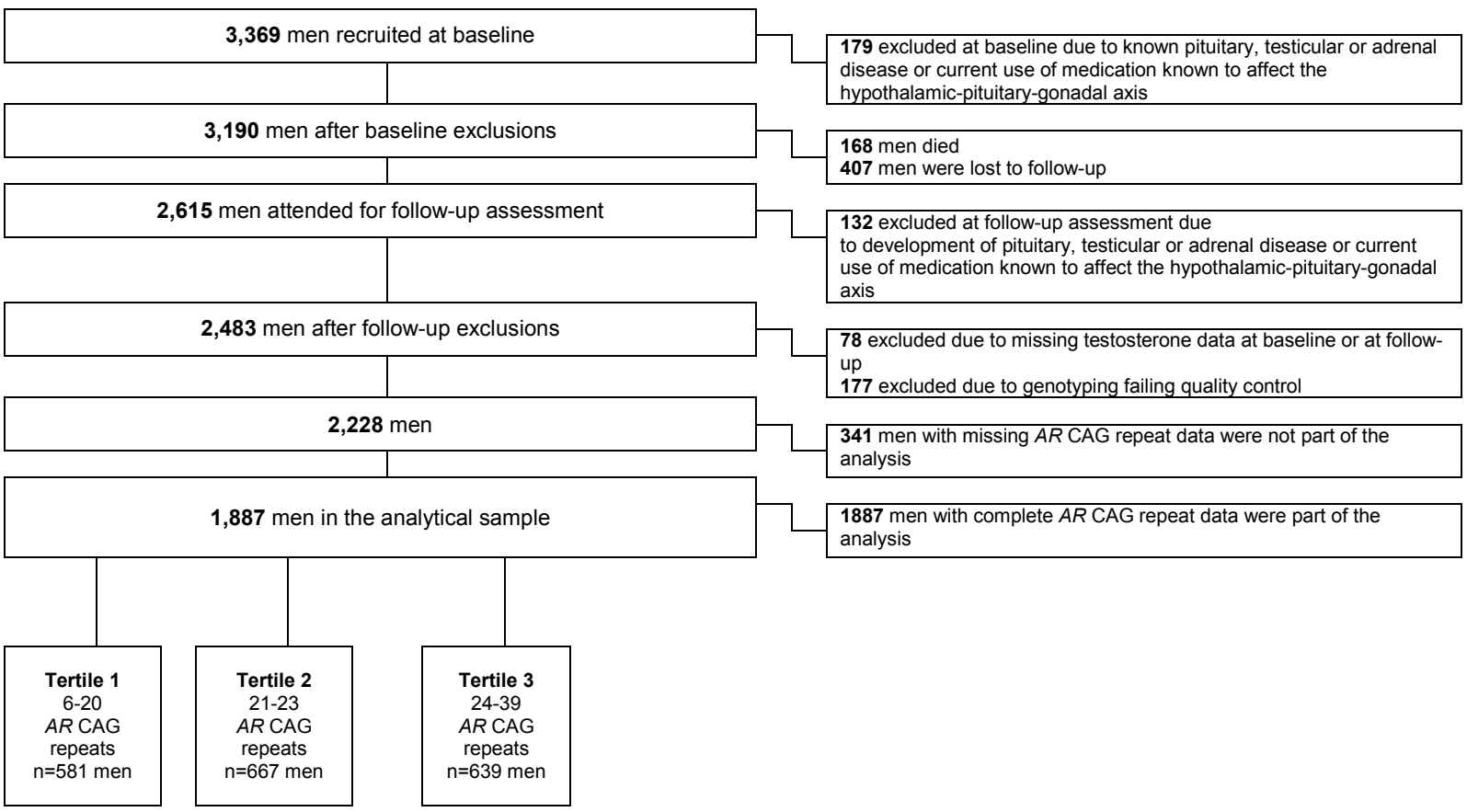

Table 1. Baseline and follow-up candidate ageing-related parameters of men with complete *AR* CAG repeat data

Parameter	Baseline Men with *AR* CAG repeat data present (n=1887)	Follow-up Men with *AR* CAG repeat data present (n=1887)
Study age, (years)	58.3 ±10.5	62.7 ±10.5***
Systolic blood pressure, (mmHg)	144.4 ±19.8	146.2 ±19.7***
Diastolic blood pressure, (mmHg)	87.0 ±11.8	84.6 ±11.6***
BMI, (kg/m^2)	27.6 ±3.9	27.8 ±4.2***
Waist circumference, (cm)	98.0 ±10.6	99.5±11.3***
MUAC (cm)	27.8 ±2.6	27.2 ±2.7***
PASE	205.0 ±89.1	181.4 ±96.0***
50ft walk, (sec)	13.1 ±2.5	14.0 ±3.7***
PPT-rating	24.3 ±2.4	23.7 ±2.5***
Fasting plasma glucose, (mmol/L)	5.6 ±1.2	5.5 ±1.3
HOMA-IR	3.1 ±4.1	3.0 ±2.9**
Total cholesterol, (mmol/L)	5.6 ±1.0	5.2 ±1.1***
HDL-cholesterol, (mmol/L)	1.4 ±0.4	1.4 ±0.4***
LDL-cholesterol, (mmol/L)	3.5 ±0.9	3.2 ±1.0***
Triglycerides, (mmol/L)	1.6 ±1.2	1.5 ±2.0
PSA, (ng/mL)	1.6 ±2.6	2.1 ±6.5**
IPSS scores	5.2 ±5.7	6.3 ±6.2***
Hb, (g/L)	150.3 ±10.4	149.8 ±11.6
DSST	28.9 ±8.4	27.8 ±9.0***
US BMD, (g/cm^2)	0.6 ±0.1	0.9 ±14.9
US BUA, (dBIMHz/cm)	81.0 ±19.0	83.1 ±18.2***
US SOS, (kHz)	1552.6 ±34.2	1550.8 ±32.9***
Testosterone, (nmol/L)	17.0 ±5.9	16.6 ±6.0***
Free testosterone, (pmol/L)	305.1 ±85.7	289.6 ±86.7***
SHBG, (nmol/L)	41.6 ±18.4	44.1 ±19.7***
LH, (U/L)	5.8 ±3.8	6.2 ±4.5***
FSH, (U/L)	7.8 ±7.5	8.2 ±8.6***
TT:LH ratio	3.6 ±1.9	3.5 ±2.0***
LHxTT product	101.2 ±76.5	103.3 ±76.9
Oestradiol (pmol/L; radio-immunoassay)	91.3 ±27.9	90.6 ±35.2
Oestradiol (pmol/L; GC-MS)	73.6 ±24.9	---
E2:TT ratio	6.0 ±4.1	6.1 ±3.9
Overall sexual function	21.0 ±6.5	21.1 ±6.9***
SF36 physical function	51.1 ±7.5	50.5 ±8.1**
BDI total	6.5 ±6.1	6.2 ±6.4*
SF36 mental function	52.2 ±8.7	52.1 ±9.0
SF36 vitality	15.2 ±2.8	15.0 ±2.9*
Morning erection frequency score	3.5 ±1.9	3.4 ±1.9*
Frequency of sexual thoughts score	5.2 ±2.0	4.8 ±2.1***
Erectile function score	1.9 ±1.0	2.1 ±1.0***
Vigorous activity score	2.2 ±0.7	2.1 ±0.8**
Limited walking score	2.9 ±0.4	2.8 ±0.5***
Unable to bend score	2.7 ±0.6	2.6 ±0.6
Sadness score	4.2 ±0.9	4.3 ±0.9
Loss of energy score	0.6 ±0.6	0.6 ±0.6*
Fatigue score	0.5 ±0.6	0.5 ±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)***
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)

Data are expressed as unadjusted mean ±SD for continuous variables and number (percentages) for categorical variables.
Abbreviations: CardioVascular Disease; BDI, Beck Depression Inventory score; SF-36, medical outcome study short form 36 item questionnaire; BMI, Body Mass Index; WC, waist circumference; MUAC, Mid Upper Arm Circumference; PASE, Physical Activity Scale for the Elderly; PPT, Physical Performance Test; DSST, Digital Symbol Substitution Test; FPG, Fasting Plasma Glucose concentration; HOMA-IR, HOmeostatic Model of Insulin Resistance; PSA, serum Prostate Specific Antigen concentration; IPSS, international prostate symptom score; Hb, plasma HaemogloBin concentration; SOS, Speed of Sound; BUA, Broadband Ultrasound Attenuation. BMD, Bone Mineral Density.
Longitudinal (unadjusted) within-group differences:
* = $p < 0.05$ as assessed by paired T-test for continuous variables or McNemar test for binary variables
** = $p < 0.01$ as assessed by paired T-test for continuous variables or McNemar test for binary variables
*** = $p < 0.001$ as assessed by paired T-test for continuous variables or McNemar test for binary variables

Table 2a. Longitudinal changes in candidate androgen-sensitive parameters associated with the number of CAG repeats in the *Androgen Receptor* (linear regression)

Parameter (difference)	N	Model 1 Unadjusted	Model 2 Adjusted for age and centre	Model 3 Adjusted for age, centre and baseline total testosterone	Model 4 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)
50ft walk, (sec)	1807	0.04 (-0.01, 0.08	0.04 (-0.01, 0.08)	**0.04 (0.00, 0.09)[a]**	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)[a]**
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)

Data are expressed as standardized beta regression coefficients (95% confidence intervals).
Abbreviations: BDI, Beck Depression Inventory score; SF-36, medical outcome study short form 36 item questionnaire; BMI, Body Mass Index; WC, waist circumference; MUAC, Mid Upper Arm Circumference; PASE, Physical Activity Scale for the Elderly; PPT, Physical Performance Test; DSST, Digital Symbol Substitution Test; FPG, Fasting Plasma Glucose concentration; HOMA-IR, HOmeostatic Model of Insulin Resistance; HDL-cholesterol, High Density Lipid cholesterol; LDL-cholesterol, Low Density Lipid cholesterol; PSA, serum Prostate Specific Antigen concentration; IPSS, international prostate symptom score; Hb, plasma HaemogloBin concentration; SOS, Speed of Sound; BUA, Broadband Ultrasound Attenuation. BMD, Bone Mineral Density.
a = p<0.05, b = p<0.01 (all p>0.01)

Table 2b. Longitudinal changes in reproductive hormone levels associated with the number of CAG repeats in the *Androgen Receptor* (linear regression)

Parameter (difference)	N	Model 1 Unadjusted	Model 2 Adjusted for age and centre	Model 3 Adjusted for age, centre and baseline total testosterone	Model 4 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)
E2, (pmol/L; radio-immunoassay)	1859	0.02 (-0.02, 0.06)	0.02 (-0.02, 0.07)	0.02 (-0.03, 0.06)	---
E2: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)
LHxTT 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)

Data are expressed as standardized beta regression coefficients (95% confidence intervals).
a = p<0.05, b = p<0.01 (all p>0.01)

Table 2c. Reproductive hormone levels at follow-up associated with the number of CAG repeats in the *Androgen Receptor* (linear regression)

Parameter (Phase 2)	N	Model 1 Unadjusted	Model 2 Adjusted for age and centre	Model 3 Adjusted for age, centre and baseline total testosterone	Model 4 Adjusted for age, centre and baseline oestradiol
Total testosterone, (nmol/L)	1887	**0.07 (0.02, 0.11)[b]**	**0.07 (0.02, 0.11)[b]**	---	0.04 (-0.00, 0.08)
Free testosterone, (pmol/L)	1871	**0.08 (0.04, 0.13)[c]**	**0.08 (-0.04, 0.12)[c]**	---	**0.06 (0.02, 0.10)[b]**
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)
E2, (pmol/L; radio-immunoassay)	1865	**0.07 (0.03, 0.12)[b]**	**0.07 (0.03, 0.12)[b]**	**0.06 (0.01, 0.10)[a]**	**---**
E2: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)
LHxTT 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)

Data are expressed as standardized beta regression coefficients (95% confidence intervals)
a = p<0.05, b = p<0.01

Table 2d. 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)

Parameter (difference)	N	Model 1 Unadjusted	Model 2 Adjusted for age and centre	Model 3 Adjusted for age, centre and baseline total testosterone	Model 4 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)
SF36 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)
SF36 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)
SF36 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)

Data are expressed as standardized beta regression coefficients (95% confidence interval).
a = p<0.05, b = p<0.01 (all p>0.01)

Table 2e. Development of medical conditions associated with the number of CAG repeats in the *Androgen Receptor* (logistic regression)

Parameter (development)	N	Model 1 Unadjusted	Model 2 Adjusted for age and centre	Model 3 Adjusted for age, centre and baseline total testosterone	Model 4 Adjusted for age, centre and baseline oestradiol
Poor Health	1445	**1.05 (1.00, 1.11)[a]**	**1.05 (1.00, 1.11)[a]**	**1.06 (1.00, 1.11)[a]**	**1.05 (1.00, 1.11)[a]**
≥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)

Data are expressed as odds ratios (95% confidence intervals).
a = p<0.05, b = p<0.01 (all p>0.01)