Received: 15 April 2023 | Revised: 7 August 2023 | Accepted: 10 August 2023

DOI: 10.1111/cen.14962

ORIGINAL ARTICLE

WILEY

Number of CAG repeats and mortality in middle aged and older men

Adrian Heald[1,2] | Michael J. Cook[3,4] | Leen Antonio[5,6] | Jos Tournoy[7,8] | Parisa Ghaffari[1] | Fahmida Mannan[2] | Helene Fachim[1,2] | Dirk Vanderschueren[5,6] | Ian Laing[9] | Geoff Hackett[10] | Felipe F. Casanueva[11] | Ilpo T. Huhtaniemi[12] | Mario Maggi[13] | Giulia Rastrelli[13] | Jolanta Slowikowska-Hilczer[14] | Fred Wu[2,15] | Terence W. O'Neill[16,17]

[1]Department of Endocrinology and Diabetes, Salford Royal Hospital, Salford, UK

[2]The School of Medicine and Manchester Academic Health Sciences Centre, University of Manchester, Manchester, UK

[3]Centre for Health Informatics, Division of Informatics, Imaging and Data Science, Manchester Academic Health Science Centre, The University of Manchester, Manchester, UK

[4]National Institute for Health and Care Research Applied Research Collaboration Greater Manchester, Manchester, UK

[5]Department of Endocrinology, University Hospitals Leuven, Leuven, Belgium

[6]Clinical and Experimental Endocrinology, Department of Chronic Diseases and Metabolism, KU Leuven, Leuven, Belgium

[7]Department of Public Health and Primary Care, Gerontology and Geriatrics Unit, KU Leuven-University of Leuven, Leuven, Belgium

[8]Department of Geriatric Medicine, University Hospitals Leuven, Leuven, Belgium

[9]Department of Biochemistry, Royal Preston Hospital, Preston, UK

[10]Aston University Medical School, Birmingham, UK

[11]Department of Medicine, Santiagode Compostela University, Complejo Hospitalario Universitariode Santiago (CHUS), CIBER de Fisiopatología Obesidady Nutricion (CB06/03), Instituto Salud Carlos III, Santiago de Compostela, Spain

[12]Department of Metabolism, Institute of Reproductive and Developmental, Digestion and Reproduction, Imperial College London, London, UK

[13]"Mario Serio" Department of Experimental and Clinical Biomedical Sciences, Endocrinology Unit, University of Florence, Florence, Italy

[14]Department of Andrology and Reproductive Endocrinology, Medical University of Łódź, Łódź, Poland

[15]Department of Endocrinology, Manchester Royal Infirmary, The University of Manchester, Manchester, UK

[16]Centre for Epidemiology Versus Arthritis, The University of Manchester, Manchester, UK

[17]Salford Royal NHS Foundation Trust, Manchester Academic Health Science Centre, Salford, UK

Correspondence
Adrian Heald, Department of Diabetes and Endocrinology, Salford Royal NHS Foundation Trust, Salford, UK; The School of Medicine and Manchester Academic Health Sciences Centre, The University of Manchester, Manchester, UK.
Email: adrian.heald@manchester.ac.uk

Abstract

Design: The androgen receptor (AR) mediates peripheral effects of testosterone. Previous data suggests an association between the number of CAG repeats in exon-1 of the AR gene and AR transcriptional activity. The aim of this analysis was to determine the association between the number of AR CAG repeats and all-cause mortality in men and the influence of testosterone level on the association.

Patients and Measurements: Follow-up data to 27 January 2018 were available for men aged 40–79 years recruited across six countries of the European Male Aging

Study between 2003 and 2005. Cox proportional hazards modelling was used to determine the association between CAG repeat number/mortality. Results were expressed as hazard ratios (HR)/95% confidence intervals (CI).

Results: One thousand nine hundred and seventy-seven men were followed up. Mean baseline age was 60 ± 11.1 years. Mean duration of follow-up was 12.2 years. At follow up 25.1% of men had died. CAG repeat length ranged from 6 to 39, with the highest proportion of CAG repeat number at 21 repeats (16.4%). In a multivariable model, compared to men with 22–23 AR CAG repeats: for men with <22 and >23 AR CAG HR, 95% CI for mortality were, <22 CAG repeats 1.17 (0.93–1.49) and >23 CAG repeats 1.14 (0.88–1.47). In a post-hoc analysis, the association was significant for men in the lowest tertile of baseline testosterone (<14.2 nmol/L) with >23 CAG repeats: in the adjusted model for <22 and >23 CAG repeats, respectively, 1.49 (0.97–2.27) and 1.68 (1.06–2.67) versus 22–23 repeats.

Conclusions: Our European-wide cohort data overall found no association of androgen receptor CAG repeat number and mortality in men. However, post hoc analysis suggested that an association might be present in men with lower baseline testosterone concentrations, which merits further investigation.

KEYWORDS
CAG, EMAS, male, mortality, testosterone

1 | INTRODUCTION

In humans, the peripheral effects of testosterone are mediated through the androgen receptor (AR), present on numerous cells integral to the neuro-endocrine axis. It has been suggested that there may be an 'optimal' number of CAG repeats, relating to AR-mediated transcriptional activity and consequent downstream effects in metabolically active tissues and that men with a number of CAG repeats above or below the optimum may possibly experience less-favourable long-term outcomes.

Based on data from the Manchester arm of the population-based European Male Aging Study (EMAS), we recently reported a weak association between the number of AR CAG repeats and the risk of mortality in older men.[1] This mirrored to some extent the findings of an earlier cohort study of men with type 2 diabetes (T2D) that a 'U' shaped curve relation exists between number of CAG repeats and mortality in men.[2] There was a suggestion also that the strength of the association between CAG repeat and mortality was greater among those with a lower testosterone level.

The aim of this analysis was to assess the association between number of AR CAG repeats and mortality in a larger cohort of men recruited to the EMAS study and to explore the influence of testosterone level on the association.

2 | MATERIALS AND METHODS

Participants were recruited in the European Male Aging Study between 2003 and 2005.[3,4] EMAS is a multicentred prospective cohort study evaluating the prevalence, incidence and geographical distribution of gender-specific and general symptoms of ageing in men, including their endocrine, genetic and psychosocial predictors. Men aged 40–79 years were recruited from eight participating countries in Europe (Italy, Belgium, Poland, Sweden, United Kingdom, Spain, Hungary and Estonia). Men with known hypothalamic/pituitary disease and those on androgen or anti-androgen treatments were excluded.

For the purposes of this analysis follow-up mortality data were available on men from six out of the eight participating countries. The censor date was 27 January 2018, since this was the latest date of death recorded across all six cohorts. The centres included in this analysis were Italy (Florence), Poland (Lodz), Belgium (Leuven), the United Kingdom (Manchester), Spain (Santiago de Compostela) and Estonia (Tartu).

Participating men were invited to complete a baseline questionnaire and provide a fasting venous blood sample between 2003 and 2005.

Mortality was determined up to 27 January 2018. There was some variation in the identification of mortality, based on local healthcare organisation and the availability and accessibility of national mortality registers. Several methods were used to retrieve

mortality data: national mortality registers, contacting the general practitioner of the participant, contacting the participant or a relative specified by the participant at recruitment. The specific cause of death was not available.

Ethical permission for the study was granted by Ethics Committees in each of the participating countries. All participants provided written informed consent.

2.1 | Hormones and biochemistry

A single fasting morning (venous blood sample collected before 10:00 AM) was obtained from each participant at baseline.

Testosterone (T) level was measured by liquid chromatography-mass spectrometry (LC-MS).[5] Sex Hormone Binding Globulin (SHBG) was measured by the Modular E170 platform electrochemiluminescence immunoassay (Roche Diagnostics). Free T levels were derived from total T, SHBG and albumin concentrations by the Vermeulen formula.[6] Measurement of total estradiol was carried out by gas chromatography-tandem mass spectrometry, as described previously.[7]

2.2 | Determination of CAG repeat number

Genetic analysis was completed in 2008. DNA extracted from whole blood was subjected to polymerase chain reaction (PCR) to amplify the region of the AR gene containing AR exon 1 CAG trinucleotide repeat. Details of PCR preparation, primers and conditions are described elsewhere as part of a previous study.[3] 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 labelled PCR. Ten nanograms of DNA were amplified in reactions containing 2.5 pmol each of fluorescently labelled 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 s, 55°C for 30 s, and 72°C for 30 s; 20 cycles of 89°C for 20 s, 55°C for 30 s, and 72°C for 30 s; and finally, 72°C for 10 min. Samples were then run on an ABIPRISM 3100 Genetic Analyser (Applied Biosystems) and genotyped using Genescan (Applied Biosystems). Allele frequencies were checked for consistency with HapMap data or literature where possible.

2.3 | Statistical analysis

Cox regression was used to determine the association between number of CAG repeats and all-cause mortality. Participants contribute person-time from the date of recruitment and stopped contributing at their date of death, or 27 January 2018. Based on previously published thresholds,[8] CAG repeats were categorised as <22, 22–23 (reference group) and >23. Analyses were adjusted for age at recruitment total testosterone, sex hormone binding globulin (SHBG) and estradiol at baseline.

To determine whether the association between AR CAG repeats and mortality varied by level of total testosterone, we categorised total testosterone into tertiles of baseline testosterone and repeated the Cox regression analysis for participants in each of the three tertiles of total testosterone. In the models stratified by tertile of total testosterone, we adjusted for total testosterone as a continuous variable.

3 | RESULTS

3.1 | Descriptive characteristics

In total 1977, men were recruited to the study who had both CAG repeat analysis and mortality data. The mean (standard deviation) age range at recruitment of the 1977 men who contributed data for the analysis was 60 ± 11.1 years. Mean duration of follow-up was 12.2 years with maximum 14.4 years. Mean baseline total testosterone level was 17.2 ± 6.2 nmol/L. The distribution of baseline serum total testosterone is shown in Figure 1. During a follow-up period, of up to 14.4 years 497/1977 (25.1%) of men had died. Baseline data are summarised in Table 1. Further details of the cohort can be found in Pye et al.[3]

3.2 | CAG repeat distribution

In this group, the highest proportion of CAG repeats occurred for the 21 repeating motif in 325/1977 = 16.4% of individuals). The number of CAG repeats varied between 6 and 39 (Table 2).

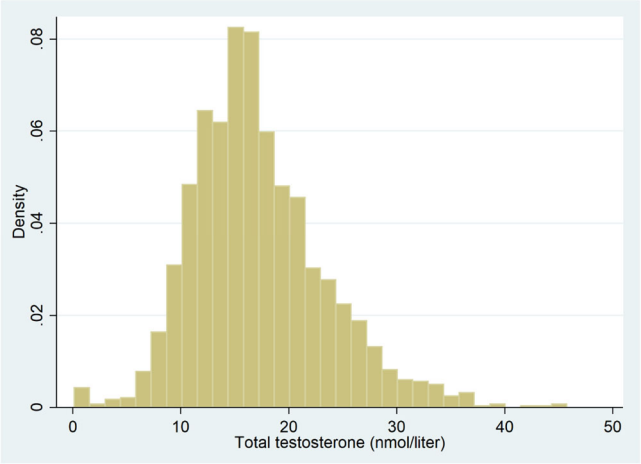

FIGURE 1 Distribution of baseline serum testosterone.

TABLE 1 Participant characteristics.

	Mean (SD)
Age at recruitment (years)	60.1 (11.1)
Total testosterone (nmol/l)[a]	17.2 (6.2)
Total oestradiol (pmol/l)[b]	75.1 (25.8)
Sex hormone-binding globulin (nmol/l)[c]	43.0 (19.4)

[a]Data on total testosterone available for 1972 men (99.7% of the cohort).
[b]Data on total oestradiol available for 1950 men (99% of the cohort).
[c]Data on sex hormone-binding globulin available for 1972 men (99.7% of the cohort).

3.3 | Relation between CAG repeat number and mortality

Modelling of CAG number as a restricted cubic spline gave the CAG repeat number 22–23 as associated with the lowest hazard ratio (HR) for death in the men followed up. Compared with participants in the reference range of 22–23 CAG repeats, participants with <22 and >23 CAG repeats, HR for <22 CAG was 1.17 (0.93–1.49) and for >23 CAG repeats was 1.14 (0.88–1.47) (Table 3) for all cause mortality. Figure 2 shows Kaplan–Meier curve for the unadjusted analysis of CAG repeat number versus mortality for all 1977 men.

The relation between AR CAG repeats and mortality varied by tertile of total testosterone at baseline (Table 4). Among those with a testosterone level in the lower tertile (<14.2 nmol/l), in an unadjusted model the HR for death among those with <22 and >23 CAG repeats (compared to reference group of 22–23 CAG repeats) was 1.49 (0.99–2.25) and 1.71 (1.10–2.68), respectively. In a model adjusted for age at recruitment, total testosterone (as a continuous measure), total estradiol and SHBG the HR was (compared to reference group of 22–23 CAG repeats) 1.49 (0.97–2.27) and 1.68 (1.06–2.67) for <22 and >23 CAG repeats respectively. Thus the hazard ratio only reached statistical significance for men with >23 CAG repeats in the lowest tertile of testosterone in both unadjusted and adjusted models. A similar augmented relation was found for men in the lowest tertile of circulating free testosterone.

Among those in the middle and upper tertile of testosterone the HRs were lower and nonsignificant. Substitution of free testosterone for total testosterone in the models did not materially change the results. Adjustment for sex hormone binding globulin (SHBG) and/or estradiol did not materially change the results.

Our previous results were based on data from a single centre (Manchester).[1] We looked separately in this analysis at the association between the number of CAG repeats and mortality in the Manchester cohort and in the other five EMAS centres included in this study. In the Manchester cohort, and using data on mortality to the censor date (January 2018) compared to men with 22–23 CAG repeats, the HR (95% CI) for mortality among men with <22 and >23 CAG repeats, respectively was 1.54 (0.67–3.52) and 1.63 (0.64–4.18) in a model adjusted for age at recruitment, total testosterone, total

TABLE 2 Distribution of CAG repeats.

Number of CAS repeats	*n* (%)
6	1 (0.05)
7	1 (0.05)
10	1 (0.05)
11	1 (0.05)
12	2 (0.1)
14	9 (0.46)
15	8 (0.4)
16	22 (1.11)
17	20 (1.01)
18	101 (5.11)
19	191 (9.66)
20	254 (12.85)
21	325 (16.44)
22	179 (9.05)
23	207 (10.47)
24	244 (12.34)
25	173 (8.75)
26	98 (4.96)
27	58 (2.93)
28	27 (1.37)
29	28 (1.42)
30	13 (0.66)
31	3 (0.15)
32	4 (0.2)
33	5 (0.25)
34	1 (0.05)
39	1 (0.05)

TABLE 3 Association between number of CAG repeats and all-cause mortality.

Number of CAG repeats	Hazard ratio for mortality (95% confidence interval)		
	Unadjusted	Model 1	Model 2
<22	1.17 (0.93–1.49)	1.20 (0.94–1.53)	1.16 (0.91–1.48)
22–23	Reference		
>23	1.14 (0.88–1.47)	1.14 (0.88–1.48)	1.10 (0.85–1.44)

Note: Model 1 is adjusted for age at recruitment, total testosterone, total oestradiol. Model 2 is additionally adjusted for sex hormone-binding globulin.

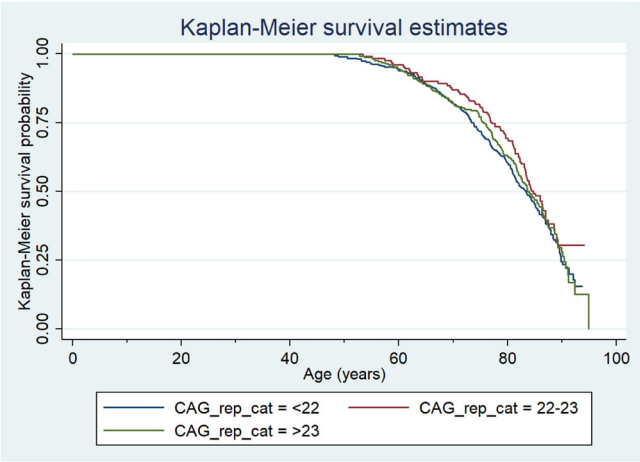

FIGURE 2 Kaplan–Meier plot showing cumulative mortality in each CAG category: <22 CAG repeats; 22–23 CAG repeats; >23 CAG repeats. The *x*-axis is age in years at death.

TABLE 4 Association between number of CAG repeats and all-cause mortality within each tertile of total testosterone.

Number of CAG repeats	Hazard ratio for mortality (95% confidence interval)		
	Unadjusted	Model 1	Model 2
Men in lowest tertile of total testosterone (T ≤ 14.175 nmol/l)			
<22	1.49 (0.99–2.25)	1.53 (1.01–2.33)	1.49 (0.97–2.27)
22–23	Reference		
>23	1.71 (1.10–2.68)	1.70 (1.07–2.69)	1.68 (1.06–2.67)
Men in middle tertile of total testosterone (14.175 nmol/l<T ≤ 18.877 nmol/l)			
<22	1.12 (0.74–1.69)	1.11 (0.73–1.69)	1.07 (0.69–1.63)
22–23	Reference		
>23	0.76 (0.48–1.20)	0.74 (0.47–1.18)	0.72 (0.45–1.15)
Men in highest tertile of total testosterone (T > 18.877)			
<22	0.94 (0.61–1.45)	0.98 (0.63–1.51)	0.96 (0.62–1.49)
22–23	Reference		
>23	1.12 (0.72–1.76)	1.11 (0.71–1.74)	1.08 (0.69–1.70)

Note: Model 1 is adjusted for age at recruitment, total testosterone, total oestradiol. Model 2 is additionally adjusted for sex hormone-binding globulin.

estradiol and sex hormone-binding globulin. In the other five centres included in this study combined (excluding Manchester), compared to men with 22–23 CAG repeats, the HR (95% CI) for mortality among men with <22 and >23 CAG repeats, respectively was 1.45 (0.89–1.48) and 1.09 (0.83–1.43).

In both the Manchester cohort and in the other five centres combined, the association between the number of CAG repeats and mortality was strongest among men in the lowest tertile of total testosterone. In the Manchester cohort, among men in the lowest tertile of total testosterone, compared to men with 22–23 CAG repeats, the HR (95% CI) for mortality among those with <22 and >23 CAG repeats, respectively was 3.44 (0.72–16.30) and 4.68 (0.88–24.92) in a model adjusted for age at recruitment, total testosterone, total estradiol and sex hormone-binding globulin. In the other five centres combined, among men in the lowest tertile of total testosterone, compared to men with 22–23 CAG repeats, the HR (95% CI) for mortality among men with <22 and >23 CAG repeats, respectively was 1.41 (0.91–2.20) and 1.56 (0.96–2.53) respectively.

4 | DISCUSSION

In this prospective study using data from the EMAS cohort, compared to those with a CAG repeat number of 22 or 23, among those, however, in the lower tertile of testosterone level at baseline, compared to those with CAG repeat number of 22 or 23 there was a significant increase in mortality among those >23 CAG repeats. In the cohort as a whole, there was no significant association.

We did not see an association of CAG repeat number with mortality expect in men in the lowest tertile of testosterone, in contrast to a previous study looking at the association between CAG repeat number and mortality in a cohort of men with Type 2 Diabetes (T2D).[2] The reason for this is unclear though one can speculate that the relative resistance to testosterone action thought to associate with a higher or lower number of CAG repeats may interact in such a way with the insulin resistance of T2D, so as to compound the consequences of insulin resistance.[9]

Independent associations between AR exon 1 CAG length and adverse cardiovascular risk factors, such as high LDL-cholesterol,[10] low HDL-cholesterol,[11] and high blood pressure[3,11] have been demonstrated by other studies. Specifically, the association between AR exon 1 longer CAG repeat length and low total testosterone concentrations appears to exert an adjunctive worsening effect on the metabolic profile.[12,13] This suggests some level of complexity of the role of the CAG polymorphism in regulating the relation between androgen effects and cardiovascular risk factors, which may inform future risk stratification when considering CAG repeat number.

Page et al.[14] reported that CAG repeat length did not predict changes in lipid variables over a 14.4-year period of follow-up, suggesting that the examined cardiovascular risk factors may not be modulated by the AR polymorphism. This previous study, however, focused on coronary heart disease whereas our study relates to all case mortality.

Many studies have reported association between low circulating testosterone and increased cardiovascular risk in men together with potential benefit of testosterone replacement.[14,15] However the literature on the interaction between CAG repeat number and serum testosterone is limited. Haring et al.[13] reported that there was no direct association between CAG repeat length and cardiometabolic

risk factors in cross-sectional and longitudinal multivariable linear regression analyses. However, men with longer CAG repeat length and low testosterone concentrations showed the highest risk of incident metabolic syndrome (relative risk: 1.51; 95% CI: 1.05–2.16). CAG repeat length was found to be a risk factor for incident low testosterone concentrations and a contributing factor to testosterone-related cardiometabolic effects. The authors recommended further investigation into the possible added clinical value of a combined assessment of CAG repeat length and serum testosterone concentrations.

Regarding the underlying biological reasons for the effects seen here, the AR gene is composed of eight exons and is located on X chromosome at q11-q12. Exon 1 of the AR gene contains a polymorphic sequence of CAG repeats, which usually varies in number from 10 to 35, and which encodes polyglutamine stretches of the AR transactivation domain.[16] The evidence suggests that the CAG number is negatively correlated with the transcriptional activity of the AR[17,18] which accords with what we report in relation to the higher number of CAG repeats >23 being associated with a higher mortality rate in men with low total testosterone.[19]

There are a number of limitations which need to be considered when interpreting the data. Our findings related to all cause (not cause-specific) mortality; we did not have any information about cause-specific mortality in our analysis and further work is needed to look at potential associations between CAG repeat number and specifically cardiovascular mortality as well as other causes of death. However, the reporting system for mortality was robust.

In our analysis, we looked at the association between CAG repeat length and mortality stratified by tertile of testosterone. The lower tertile threshold (14.2 nmol/L) was within the currently accepted 'normal' range. It is a limitation that small numbers precluded more detailed assessment of risk among those with lower testosterone levels and that we only had a single measurement of testosterone. Further studies are needed more precisely to define the interaction between CAG repeat number and circulating testosterone concentration in modulating mortality risk in men. Finally, our data relate to a predominantly Caucasian population and should not be generalised.

5 | CONCLUSIONS

Our European-wide cohort data suggests among those with the lowest tertile of baseline testosterone an association between AR CAG repeat number and mortality in men. A greater understanding of the interaction between CAG repeat number and circulating testosterone level may further our understanding of the endocrine processes that modulate mortality risk in men as they age, with future implications for risk stratification in this cohort of patients. We accept that our findings are exploratory, while they do provide the basis for further studies.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Adrian Heald http://orcid.org/0000-0002-9537-4050
Leen Antonio http://orcid.org/0000-0002-1079-2860

REFERENCES

1. Heald A, Cook M, Antonio L, et al. The number of androgen receptor CAG repeats and mortality in men. *Aging Male*. 2022;25:167-172.
2. Heald AH, Yadegar Far G, Livingston M, et al. Androgen receptor-reduced sensitivity is associated with increased mortality and poorer glycaemia in men with type 2 diabetes mellitus: a prospective cohort study. *Cardiovasc Endocrinol Metab*. 2020;10:37-44.
3. Pye SR, Huhtaniemi IT, Finn JD, et al. EMAS Study Group. Late-onset hypogonadism and mortality in aging men. *J Clin Endocrinol Metab*. 2014;99:1357-1366.
4. Lee DM, O'Neill TW, Pye SR, et al. EMAS study group. The European Male Ageing Study (EMAS): design, methods and recruitment. *Int J Androl*. 2009;32:11-24.
5. Ketha H, Kaur S, Grebe SK, Singh RJ. Clinical applications of LC-MS sex steroid assays: evolution of methodologies in the 21st century. *Curr Opin Endocrinol Diab Obes*. 2014;21:217-226.
6. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab*. 1999;84:3666-3672.
7. doi:10.1016/j.jsbmb.2006.02.004. Accessed January 5, 2023.
8. 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. *Int J Androl*. 2011;34:327-332.
9. Gianatti EJ, Grossmann M. Testosterone deficiency in men with type 2 diabetes: pathophysiology and treatment. *Diabetic Med*. 2020;37:174-186.
10. Trzmiel-Bira A, Filus A, Kuliczkowska-Płaksej J, et al. The CAG repeat polymorphism in androgen receptor gene repeat and frequency of chosen parameters of metabolic syndrome in 45-65 aged men in Wroclaw population. *Endokrynol Pol*. 2008;59:477-482.
11. 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.
12. 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. *J Clin Endocrinol Metab*. 2007;92:3844-3853.
13. Haring R, Ernst F, Schurmann C, et al. The androgen receptor CAG repeat polymorphism as a risk factor of low serum testosterone and its cardiometabolic effects in men. *Int J Androl*. 2012;35:511-520.
14. Page ST, Kupelian V, Bremner WJ, McKinlay JB. The androgen receptor gene CAG repeat polymorphism does not predict increased risk of heart disease: longitudinal results from the Massachusetts male Ageing Study. *Clin Endocrinol*. 2006;65:333-339.
15. Hackett G, Heald AH, Sinclair A, Jones PW, Strange RC, Ramachandran S. Serum testosterone, testosterone replacement therapy and all-cause mortality in men with type 2 diabetes: retrospective consideration of the impact of PDE5 inhibitors and statins. *Int J Clin Pract*. 2016;70:244-253.
16. Stanworth RD, Kapoor D, Channer KS, Jones TH. Androgen receptor CAG repeat polymorphism is associated with serum testosterone

levels, obesity and serum leptin in men with type 2 diabetes. *Eur J Endocrinol*. 2008;159:739-746.
17. Tirabassi G, Cignarelli A, Perrini S, et al. Influence of CAG repeat polymorphism on the targets of testosterone action. *Int J Endocrinol*. 2015;2015:1-12.
18. Stanworth RD, Kapoor D, Channer KS, Jones TH. Dyslipidaemia is associated with testosterone, oestradiol and androgen receptor CAG repeat polymorphism in men with type 2 diabetes. *Clin Endocrinol*. 2011;74:624-630.
19. Kelly DM, Jones TH. Testosterone and cardiovascular risk in men. *Front Horm Res*. 2014;43:1-20.

How to cite this article: Heald A, Cook MJ, Antonio L, et al. Number of CAG repeats and mortality in middle aged and older men. *Clin Endocrinol (Oxf)*. 2023;99:559-565. doi:10.1111/cen.14962