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SHORT REPORT
Cardiovascular genetics

Androgen receptor polymorphism, testosterone levels, and prognosis in patients with acute myocardial infarction

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Aims Low testosterone has been associated with cardiovascular disease in men but with contradictory findings. Testosterone bind to the androgen receptor and polymorphisms of the receptor gene such as CAG repeat length may affect transcriptional activity, possibly mitigating testosterone effects. The aims were to study the CAG repeat length and testosterone levels at four time points following a myocardial infarction (MI) and to analyse possible relationships between CAG repeat length and cardiovascular prognosis.

Methods and results Male patients admitted for acute MI ($n = 122$) from the Glucose in Acute Myocardial Infarction study were included. Blood samples were drawn at four time points (day after admission, at discharge, and at 3 and 12 months post-infarction) for assessment of testosterone levels. Patients were followed for a median of 11.6 years. Cox regression analyses were performed for CAG repeat length by one unit increment and by > vs. ≤median for cardiovascular events and all-cause mortality. Median CAG repeat length was 20. There was no difference in testosterone levels at each time point when dividing the cohort into ≤ vs. >CAG repeat median (=20). There was no association between CAG repeat length either as a continuous or categorical variable in unadjusted and age-adjusted Cox analyses for cardiovascular events. While CAG >20 was associated with all-cause mortality in unadjusted analyses (hazard ratio 2.19, 95% confidence interval 1.13–4.22; $P = 0.02$), it did not remain significant following adjustment for age.

Conclusion CAG repeat length was not associated with testosterone levels or prognosis in men with acute MI.

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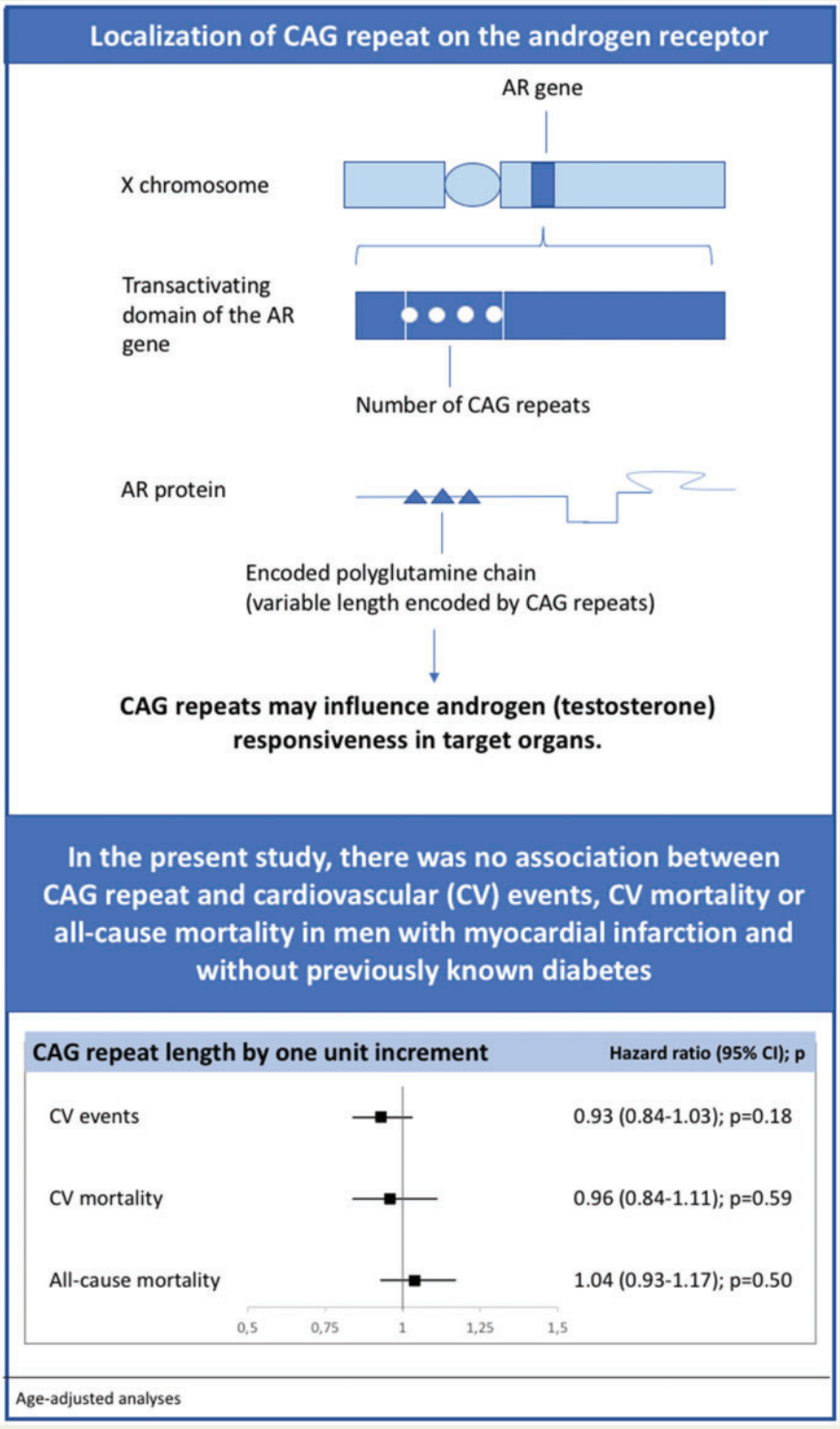
Graphical Abstract



Keywords Testosterone • Androgen receptor • Diabetes • Genetics • Cardiovascular disease

Introduction

Low testosterone in men relates to cardiovascular (CV) risk factors such as type 2 diabetes (T2DM) and increased CV morbidity.[1] Testosterone exerts its androgen effects primarily by binding to the androgen receptor (AR). The AR gene is located on the X chromosome and the transactivating domain (exon 1) of the gene has polymorphic triplets of CAG repeats encoding a variable length polyglutamine chain.[2] Androgen receptor polymorphisms may influence androgen responsiveness explaining phenotype variability besides testosterone levels.[2]

The number of CAG repeats is inversely correlated to transcriptional AR activity and androgen effects,[2] thus it may influence testosterone levels and CV risk. The aims were to examine the relationship between CAG repeat length and testosterone at four time points during the first year post-hospitalization in men with myocardial infarction (MI) and to investigate the prognostic impact of CAG repeat length.

Materials and methods

The Glucose in Acute Myocardial Infarction (GAMI) study was an observational investigation of the prevalence and prognostic impact of dysglycaemia in 181 patients (males 69%, $n = 123$) with MI without diabetes.[3,4] Blood samples were drawn on the morning after hospital admission, at discharge and after 3 and 12 months. Oral glucose tolerance test was carried out before discharge to categorize patients as having normal or abnormal glucose tolerance (newly detected diabetes mellitus or impaired glucose tolerance). Blood samples were stored at -70°C. Patients were followed for 11.6 years. Information on CV events and mortality were obtained from hospital records and the Swedish National Death Registry. The present cohort comprises male participants with blood samples available for DNA extraction ($n = 122$). The primary endpoint was a major CV event (first of CV death or non-fatal MI, stroke, or hospitalized heart failure). Secondary endpoints were CV and all-cause mortality.

Testosterone, luteinizing hormone, and sex hormone-binding globulin (SHBG) were analysed in fasting blood from the day after hospital admission ($n = 122$), at discharge ($n = 113$) and 3 ($n = 99$) and 12 ($n = 85$) months post-discharge. Testosterone was determined using liquid chromatography–mass spectrometry (LC/MS). Luteinizing hormone and SHBG were measured using ELISA. Genomic DNA was extracted with QIAamp DNA Mini Kit from whole blood and the CAG repeat length in the first exon of the AR gene on the X chromosome was amplified from genomic DNA by PCR. The following primers flanking the CAG repeats were used for amplification: 5′-FAM6-TCC AGA ATC TGT TCC AGA GCG TGC-3′ and 5′-GCT GTG AAG GTT GCT GTT CCT CAT-3′. PCR was performed on a GeneAmp 9700 thermocycler (Applied Biosystems) and PCR-FAM amplicons were resolved with capillary electrophoresis and identified with an ABI 3730 Genetic Analyzer (Applied Biosystems). CAG repeat length was determined using GeneScanTM-500LIZ® Size standards (Applied Biosystems) with GeneMapper Software (Applied Biosystems).

The GAMI study and the additional DNA analyses were approved by the local ethics committee.

Statistics

Data are presented as median (interquartile range) for continuous variables and number (percentage) for categorical variables. The Mann–Whitney *U* test (continuous variables) and χ^2 test (categorical variables) were used to test differences between groups. CAG repeat was analysed as a continuous and categorical variable divided as below and equal to or above the median level. Cox regression was used to estimate the hazard of CAG repeat by one unit increment and the respective endpoints. Model A was unadjusted and Model B was adjusted for age. Statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

The median CAG repeat length was 20. *Table 1* presents baseline characteristics with the population presented in total and dichotomized into repeat lengths CAG ≤20 and >20. In the complete population 65% had abnormal glucose tolerance. The CAG ≤20 group had lower capillary blood glucose at admission compared to the CAG >20 group (6.0 vs. 7.1 mmol/L; $P = 0.0016$), otherwise the two groups were similar. Over the course of time up to 1 year post-infarction, there was no difference in testosterone levels between those with CAG ≤20 and CAG >20 (*Table 2*). During follow-up, 50 (41%) patients experienced a CV event and 37 (30%) died [whereof 25 (=68%) from CV causes]. In the CAG ≤20 group, 40% experienced a CV event compared to 43% in the CAG >20 group ($P = 0.68$), 18 vs. 25% ($P = 0.38$) CV death, and 22% vs. 42% ($P = 0.02$) death from all causes. In Cox analyses (one unit increment), CAG repeat length was not associated with any endpoint (*Figure 1A*). In Cox analyses by CAG >20 group vs. CAG ≤20 group, CAG >20 related to increased all-cause mortality (hazard ratio 2.19, 95% confidence interval 1.13–4.22; $P = 0.02$) in unadjusted analyses. This association was attenuated in the age-adjusted analyses (*Figure 1B*). The dichotomized CAG variable did not relate to any CV endpoint.

Discussion

The main findings were that CAG repeat length did not correlate to testosterone levels at four time points up to 1 year following hospitalization and that CAG repeat length was not associated with CV events. Although all-cause mortality was higher in patients with CAG repeat length above median, it was nominally but not significantly associated after age adjustment.

Previous information regarding CAG repeat and testosterone are inconclusive. A correlation was found between the highest quartile of CAG and bioavailable testosterone but not with total testosterone.[5] In line with the present study, there was no difference in testosterone levels between men with CAG ≤21 or >21 in a Norwegian study comprising 172 men.[6] Moreover, men with more components of the metabolic syndrome tended to have a shorter CAG repeat length than healthy controls.[6] Such association may explain why the CAG repeat length in the present population (66% dysglycaemic) was slightly lower than the expected of a Caucasian population (20 vs. 21–22).[2] Low testosterone has been related to increased CV and all-cause mortality but reports remain contradictory.[1] In the current cohort testosterone predicted CV and all-cause mortality in healthy controls

Table 1 Baseline characteristics of the study participants

	ALL ($n = 121$)	CAG ≤20 ($n = 68$)	CAG >20 ($n = 53$)	*P*-value
Clinical characteristics				
Age (years)	61 (56–71)	61 (56–68)	62 (56–73)	0.43
Current smokers	38 (32)	19 (28)	19 (36)	0.32
BMI (kg/m^2)	26 (24–29)	26 (24–29)	26 (24–28)	0.34
Family history of T2DM	21 (18)	13 (19)	8 (16)	0.60
Family history of IHD	60 (50)	38 (57)	22 (42)	0.12
Previous disorders				
Myocardial infarction	26 (21)	16 (24)	10 (19)	0.54
Hypertension	32 (26)	21 (31)	11 (21)	0.21
Hyperlipidaemia	17 (14)	10 (15)	7 (13)	0.81
Pharmacological treatment				
β-blockers	34 (28)	19 (28)	15 (28)	0.97
ACE inhibitors	10 (8)	7 (10)	3 (6)	0.36
Statins	12 (10)	7 (10)	5 (9)	0.88
Biochemical characteristics				
CAG repeat length	20 (18–22)			
Capillary blood glucose (mmol/L)	6.2 (5.6–7.4)	6.0 (5.4–7.0)	7.1 (5.9–7.8)	0.002
HbA1c (%)	4.9 (4.5–5.3)	4.9 (4.6–5.3)	4.9 (3.8–4.9)	0.95
CRP (mg/L)	12.3 (4.7–26.9)	11.4 (5.5–25.3)	15.8 (4.3–37.1)	0.72
Creatinine (μmol/L)	96 (86–106)	95 (86–106)	99 (85–111)	0.65
Normal glucose tolerance[a]	41 (35)	25 (38)	16 (32)	0.51
Testosterone (ng/dL)	248 (187–346)	226 (177–334)	265 (207–383)	0.12
Free testosterone (ng/dL)[b]	0.11 (0.08–0.13)	0.09 (0.07–0.12)	0.11 (0.09–0.15)	0.06
Prevalence of low testosterone[c]	77 (64)	47 (69)	30 (57)	0.16
SHBG (nmol/L)	70 (52–93)	67 (51–93)	72 (56–92)	0.85
LH (mIU/mL)	1.9 (1.1–3.4)	1.9 (1.1–3.1)	2.0 (1.3–3.5)	0.81

Continuous variables presented as median (IQR) and categorical variables presented as *n* (%).
ACE, angiotensin converting enzyme; BMI, body mass index; CAG, Cytosine-Adenine-Guanine; CRP, C-reactive protein; HbA1c, glycosylated haemoglobin; IHD, ischaemic heart disease; IQR, interquartile range; LH, luteinizing hormone; SHBG, sex hormone-binding globulin; T2DM, type 2 diabetes mellitus.
[a]Diagnosed by oral glucose tolerance test.
[b]Free testosterone was calculated using the Vermeulen formula.
[c]Low levels of total testosterone were ≤300 ng/dL.

Table 2 Total testosterone levels (ng/dL) in relation to CAG repeat group (≤ or >20) in the study population at four time points

	Day after admission ($n = 121$)	Discharge ($n = 113$)	3 months ($n = 99$)	12 months ($n = 85$)
CAG ≤20	226 (177–334)	269 (204–415)	345 (261–427)	352 (270–437)
CAG >20	265 (207–383)	369 (212–471)	359 (298–456)	393 (287–512)
P-value	0.12	0.08	0.29	0.13

Hormone levels presented as median and interquartile range.
CAG, Cytosine-Adenine-Guanine.

but not in MI patients.[7] The explanation for conflicting results may be different study cohorts and underlying health status, but CAG repeat length and its effect on AR activity has also been suggested. In a study comprising 474 men with T2DM, high testosterone was associated with increased all-cause mortality in those with a short but decreased in those with a long CAG

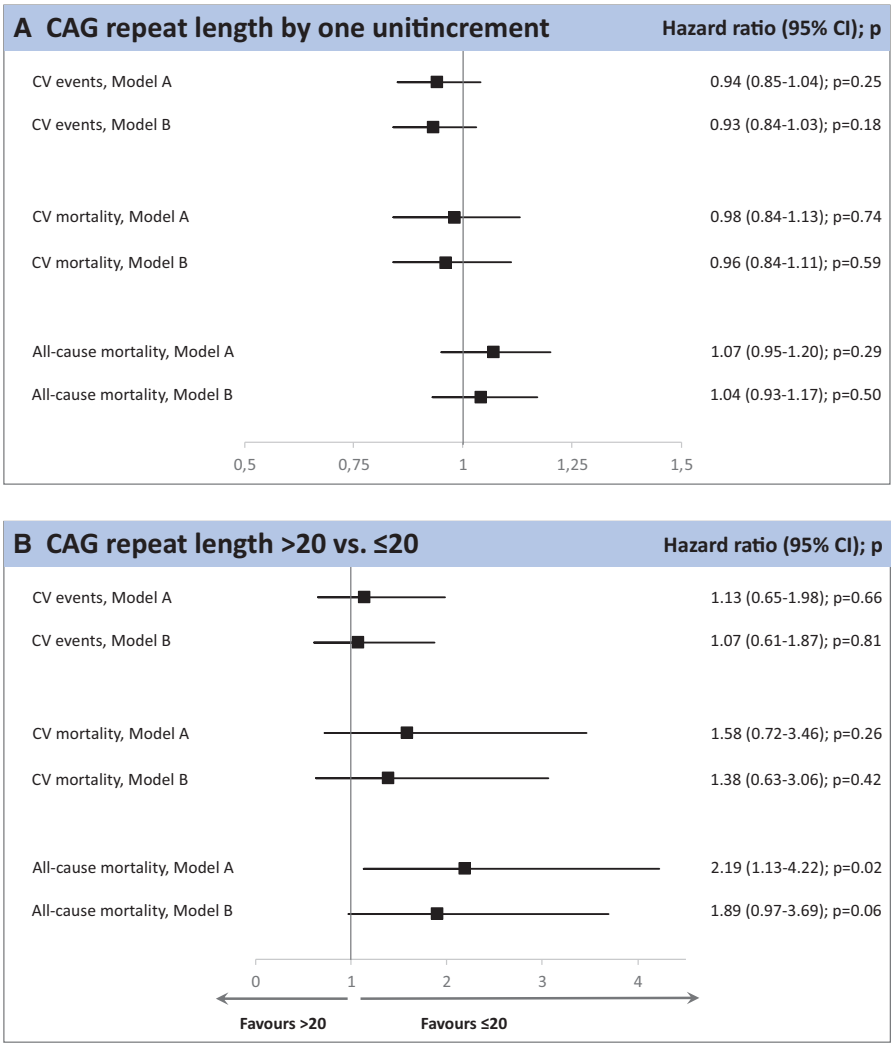


Figure 1 Hazard ratio (95% confidence interval) for the association between CAG repeat length continuously [(*A*) by increment of one unit] and categorically [(*B*) >median vs. ≤median] and cardiovascular events, cardiovascular mortality, and all-cause mortality. Model A: unadjusted. Model B: adjusted for age. CAG, Cytosine-Adenine-Guanine; CI, confidence interval; CV, cardiovascular.

repeat, suggesting that this may possibly modulate the effects of testosterone.[8] Interestingly, another study in men with T2DM showed no relation between CAG repeat length and CV events although the relation between CAG repeat length and all-cause mortality was u shaped.[9] In the present study, there was no association between CAG repeat length and CV or all-cause mortality. However, the study was limited by number of subjects, also rendering subgroup analyses difficult. Nevertheless, the present study is based on a well-defined cohort of men with MI and prospectively collected data including testosterone measurements up to a year post-infarction analyzed using LC/MS considered the ideal method of analysis. In conclusion, CAG repeat length was not related to testosterone or prognosis in men with MI highlighting the importance to further study possible mechanisms of action between testosterone and CV prognosis.

Lead author biography

Anne Wang is currently a resident in Cardiology at Karolinska University Hospital, Sweden. She started her medical studies at Karolinska Institutet in 2011 and was admitted to an MD/PhD Programme to begin her PhD in 2015. After graduation in 2017, she conducted a combined research medical internship at Karolinska University Hospital and became a licensed physician in 2019. In 2020, she defended her PhD thesis entitled 'Testosterone—of importance in patients with dysglycaemia and cardiovascular disease?' and continues to

conduct research in her area of interest, diabetes and cardiovascular disease.

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References

1. Oskui PM, French WJ, Herring MJ, Mayeda GS, Burstein S, Kloner RA. Testosterone and the cardiovascular system: a comprehensive review of the clinical literature. *J Am Heart Assoc* 2013;**2**:e000272.
2. Tirabassi G, Cignarelli A, Perrini S, Delli Muti N, Furlani G, Gallo M, Pallotti F, Paoli D, Giorgino F, Lombardo F, Gandini L, Lenzi A, Balercia G. Influence of CAG repeat polymorphism on the targets of testosterone action. *Int J Endocrinol* 2015;**2015**:298107.
3. Norhammar A, Tenerz A, Nilsson G, Hamsten A, Efendic S, Ryden L, Malmberg K. Glucose metabolism in patients with acute myocardial infarction and no previous diagnosis of diabetes mellitus: a prospective study. *Lancet* 2002;**359**:2140–2144.
4. Ritsinger V, Tanoglidi E, Malmberg K, Nasman P, Ryden L, Tenerz A, Norhammar A. Sustained prognostic implications of newly detected glucose abnormalities in patients with acute myocardial infarction: long-term follow-up of the Glucose Tolerance in Patients with Acute Myocardial Infarction cohort. *Diab Vasc Dis Res* 2015;**12**:23–32.
5. 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.
6. Skjaerpe PA, Giwercman YL, Giwercman A, Svartberg J. Androgen receptor gene polymorphism and the metabolic syndrome in 60-80 years old Norwegian men. *Int J Androl* 2008;**33**:500–506.
7. Wang A, Arver S, Flanagan J, Gyberg V, Nasman P, Ritsinger V, Mellbin LG. Dynamics of testosterone levels in patients with newly detected glucose abnormalities and acute myocardial infarction. *Diab Vasc Dis Res* 2018;**15**:511–518.
8. Wong PW, Lee HM, Lau ESH, Lim C, Ma RCW, Kong APS, Chan JCN, Luk AOY. Interactive effects of testosterone and the androgen receptor CAG repeat length polymorphism on cardiovascular-renal events and mortality in men with diabetes. *Diabetes Metab Res Rev* 2019;**35**:e3081.
9. Heald AH, Yadegar Far G, Livingston M, Fachim H, Lunt M, Narayanan RP, Siddals K, Moreno G, Jones R, Malipatil N, Rutter M, Gibson M, Donn R, Hackett G, Jones H. 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.