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The CAG trinucleotide repeat length in the androgen receptor does not predict the early onset of prostate cancer

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Objective To relate the repeat length of the androgen-receptor CAG trinucleotide to the age of onset of prostate cancer, stage and grade of disease.

Patients and methods After obtaining ethical approval, 265 patients with locally confined or locally advanced/metastatic prostate cancer were identified and evaluated for age at diagnosis (< 65 years and > 75 years). DNA was extracted from peripheral blood lymphocytes and 1 µg aliquots subjected to polymerase chain reaction using fluorescently labelled primers. Samples were then run on an ABI 377 gene scan analysis gel with an internal molecular weight marker. The length of the CAG repeat was determined by comparing the gene scan product size to samples where the CAG repeat length had been quantified using direct sequencing. The Kruskal–Wallis, Mann–Whitney and Wilcoxon two sample tests were used to analyse the data.

Results The mean (range) length of the CAG repeat in the androgen receptor was 22.2 (10–31) in the younger and 22.5 (16–32) in the older group, and was not statistically different. There was no significant association between the CAG repeat length and the age of onset of prostate cancer ($P = 0.568$) or with stage ($P = 0.577$) and grade ($P = 0.891$) of prostate cancer.

Conclusion These results suggest that there is no correlation between the androgen receptor CAG repeat length and the age of onset, stage and grade of prostate cancer, confirming recent doubts from other similar studies of a suggested correlation between shorter androgen receptor CAG repeat and early onset and aggressiveness of prostate cancer.

Keywords prostate cancer, CAG trinucleotide repeat, androgen receptor, stage, grade

Introduction

Prostate cancer is the most common urological neoplasm diagnosed in men in the USA and Western Europe [1]; 30–40% of men aged ≈ 50 years are estimated to have histological evidence of cancer of the prostate, increasing to 70–80% of men aged ≥ 80 years [2]. Despite a high prevalence of subclinical prostate cancer in young men (50 years old) only ≈ 8% progress to clinically significant disease requiring treatment [3]. Intriguingly, some men obviously live with histological evidence of prostate cancer for many years and indeed may never manifest clinical symptoms [4], whilst in others the disease is aggressive, invasive and ultimately life-threatening. The significance of subclinical prostate cancer in men of all ages is at present unclear, nor it is apparent whether men with subclinical disease would benefit from radical surgery to prevent disease progression. If this were the case, there is clearly great potential for reducing death and morbidity from prostate cancer by early intervention. At clinical diagnosis some prostate cancers cannot be cured by surgery and patients are treated with endocrine therapy; 70–80% of such patients respond favourably and disease palliation is achieved for several months or years [5]. However, whichever method of hormonal ablation is chosen, all patients with advanced disease eventually relapse with no effective second-line treatments available. For men with newly diagnosed metastatic prostate cancer, the median survival time is 12–18 months.

The modulation of androgen-regulated genes represents the most important mechanism to date for promoting the growth of prostate cancers. Current hormonal therapies are designed to prevent the activation of androgen-regulated genes by depriving the androgen receptor (AR) of its ligand, and through interference with the transactivation machinery of the receptor protein. The prostate requires androgens for normal glandular function [6,7] and both prostate cancer growth and therapy is critically dependent on the existence of a functionally normal AR [8]. Clearly changes in the activity or expression of the AR may be important in influencing the onset and severity of disease in patients with prostate cancer. Two polymorphic regions have been identified within the cod-

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ing region of exon 1 of the AR; both of these regions are trinucleotide repeats (CAG and GGC) and may influence the activity of the AR. There is a reported inverse linear relation between the CAG repeat length and the AR transactivation function [9]. In conditions such as Kennedy's disease, with long CAG repeats (> 40) the AR shows less transactivation activity [10], resulting in patients with low virilization and subfertility. Prostate cells with short CAG repeats in the AR gene would therefore have greater sensitivity to proliferative stimulation of androgens, resulting in better conditions for malignant growth [11]. Interestingly, the frequency distribution of human AR CAG repeat length in different races varies with the incidence of prostate cancer [12]. African-Americans, who have the highest reported incidence of prostate cancer, more frequently have shorter CAG repeats than Caucasians and Asian-Americans [12]; ≈ 75% of Caucasians have 19–25 CAG repeats [13]. An association between short CAG repeats and the early onset of prostate cancer has been reported [14], although other studies refute any such relationship [15]. The GGC repeat length is also thought to influence susceptibility to prostate adenocarcinoma (normal repeat length 8–17 [12]). Germ-line mutations in the AR gene may cause variation in the length of the CAG and GGC repeats, altering the individual's susceptibility to prostate cancer. However, somatic mutations might alter the AR activity within the prostate cancer cells and lead to an early escape from hormonal control or a more aggressive disease. Proof of a definite relationship between the CAG and GGC repeat lengths and prostate cancer may help clinicians to identify such young patients with a high risk of aggressive prostate cancer, and thus be able to offer them regular screening and early radical treatment.

The aim of this study was to investigate the correlation between the length of CAG repeats and the age of onset of prostate cancer; we also assessed the relationships between CAG length and prostate cancer stage and grade.

Patients and methods

Patients with histopathological evidence of prostate cancer were identified from prostate cancer clinics in the West of Scotland. In all, 265 men with prostate cancer were recruited and, depending on their age at diagnosis, were divided into two groups, i.e. 155 men with an 'early diagnosis' of prostate cancer aged ≤ 65 years were compared with 110 men with prostate cancer who were ≥ 75 years old at the time of diagnosis. The Gleason score was used to grade the tumours [16]; a Gleason score of 2–4 was taken as low grade, intermediate as 5–7 and 8–10 as high grade. The TNM classification was used to stage the tumours [17]. Apart from three patients who were of Asian ethnic origin, all patients were Caucasian. Tumour stage, grade and PSA level were recorded at the time of diagnosis for each patient (Table 1); the PSA levels were assessed at 3-monthly intervals during the study.

Table 1 The number of patients and number of mean CAG repeats in each stage and grade

	Age at diagnosis	
Variable	*<65*	*≥75*
Stage		
T1	71	48
T2	46	26
T3	24	23
T4	14	13
Grade		
2–4	56	26
5–7	70	63
8–10	29	21
Stage		
T1	22.0	22.2
T2	22.2	22.6
T3	22.0	22.7
T4	23.1	22.7
Grade		
2–4	21.5	22.2
5–7	22.6	22.5
8–10	22.5	22.7

Apart from 38 patients who were being followed by watchful waiting, all patients received some form of treatment, i.e. radical prostatectomy (42), radical radiotherapy (47) and hormonal manipulation for advanced disease either in the form of LHRH analogues or antiandrogens (126) or bilateral orchidectomy (12).

After obtaining ethical approval and patient consent, a blood sample was withdrawn from the patients. DNA was extracted from the peripheral blood leukocytes [18] and stored at – 20 °C until analysis. The AR polymorphisms were analysed using an automated fluorescent PCR; The 5′ primer was fluorescently labelled for detection on an automated DNA analyser. The primer sequence used was:

5′-TCCAGAATCTGTTCCAGAGCGTGC-3′ (forward) and 5′-CTTGGGGAGAACCATCCTCA-3′ (reverse) [19].

The PCR conditions were: 95 °C for 5 min followed by 95 °C for 1 min, 53 °C for 1 min and 72 °C for 2 min, all repeated for 30 cycles, followed by 72 °C for 20 min and 15 °C for 10 min. PCR products were analysed using an ABI 377 automated DNA analyser (Perkin Elmer Applied Biosystems Ltd, UK) using Genescan software. The PCR product size range was 270–310 bp. The length of the CAG trinucleotide repeat was determined by comparing the product size to samples where the CAG repeat length had previously been quantified using direct sequencing.

The Kruskal–Wallis, Mann–Whitney and Wilcoxon two sample test were used to analyse the data.

Results

The mean (range) length of the CAG repeat in the AR was 22.2 (10–31) in the younger and 22.5 (16–32) in the older group, with an overall mean of 22.3; there was no significant difference between the groups ($P = 0.568$; Table 1). When CAG repeat lengths in patients with prostate cancer diagnosed when ≤ 55 years old were compared with those of patients diagnosed when ≥ 75 years old, there was again no significant difference, and none between the CAG repeat length and the age of onset of prostate cancer (Fig. 1a). The mean CAG repeat length for T1 was shorter than that for T4 tumours (Table 1), but this was not significant ($P = 0.577$; Fig. 1b). Low-grade tumours (Gleason score 2–4) had slightly shorter CAG repeats than high-grade tumours (Gleason score 8–10), but again this was not significant ($P = 0.891$; Fig. 1c). There was no significant difference with stage or grade for patients with short or long CAG repeats.

Discussion

The PCR technique has been used extensively with good accuracy in molecular biology to enhance and amplify the

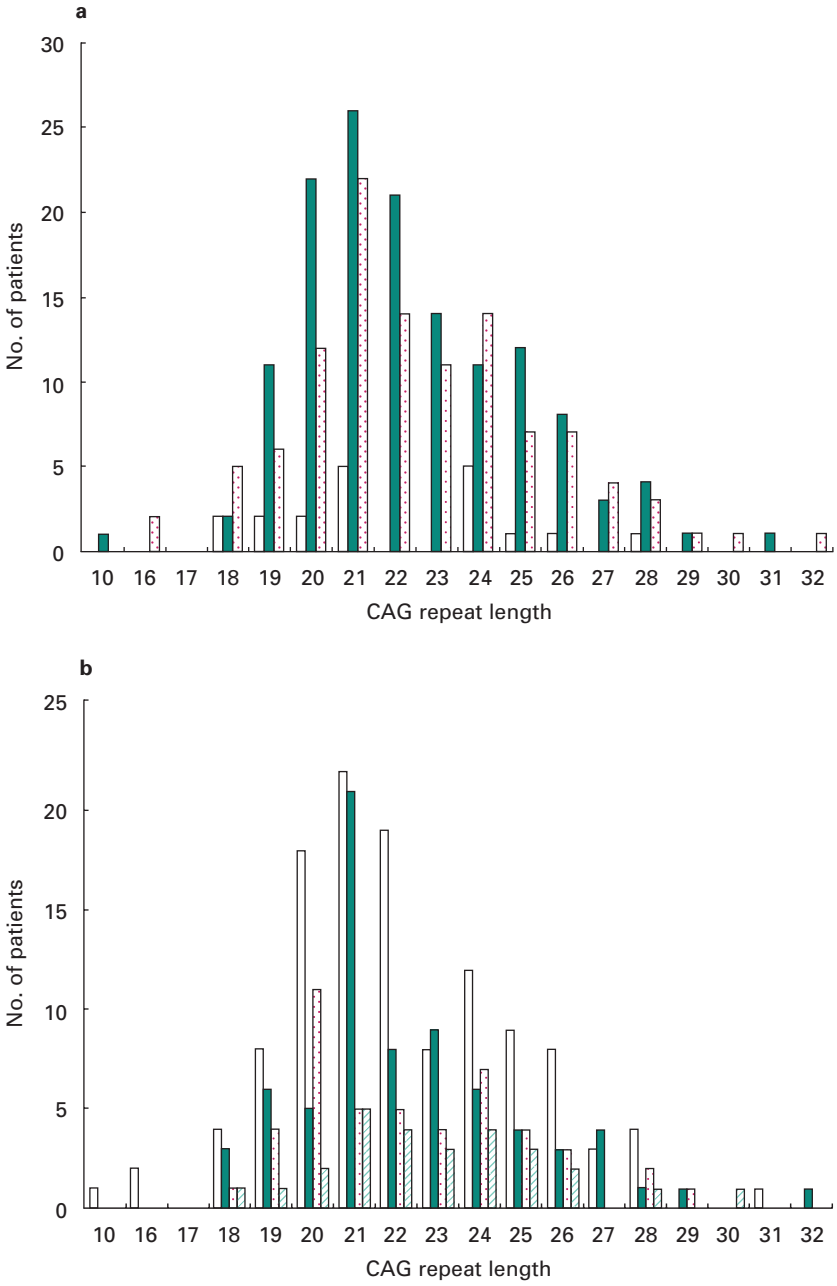

Fig. 1. The frequency distribution of CAG repeat length in the AR among: **a,** the different age groups (open bars < 55 years, green bars, < 65 years; red stipple, ≥ 75 years); **b,** T stage (open bars, T1, green bars, T2; red stipple, T3; light green dashed, T4); and **c,** Gleason grade (open bars, 1–4; green bars, 5–7; red stipple, 8–10).

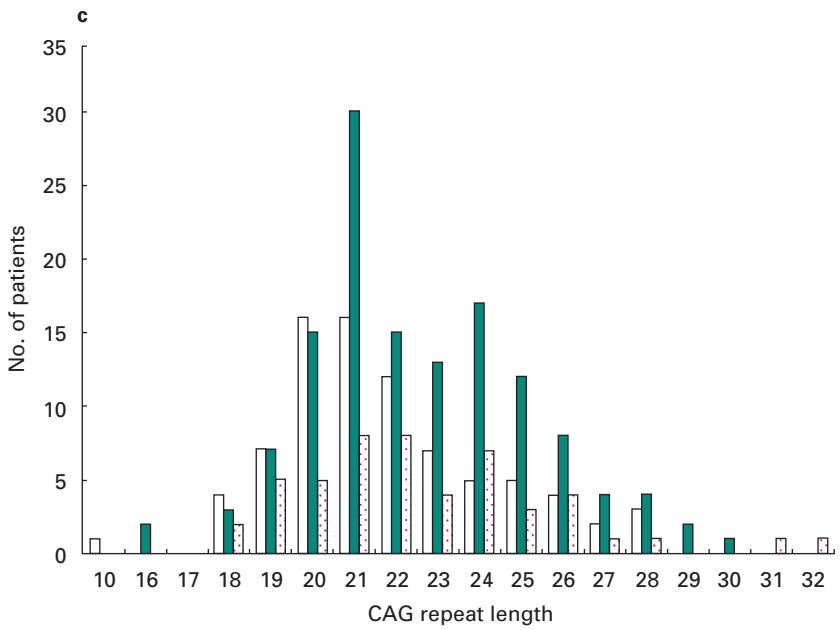

Fig. 1. (*Continued*)

DNA segment being studied; however, no single protocol is appropriate to all situations. Various problems, including low product yield, presence of nonspecific background bands, formation of 'primer-dimers' competing for amplification, and mutations or heterogeneity caused by misincorporation have been encountered. To minimize the risk of error, various modifications were used in optimizing PCR conditions, sample sequencing and allele typing [20].

The AR CAG repeat length in the DNA extracted from peripheral blood lymphocytes may be different to that within the prostate cancer cells. If so then the difference may be secondary to a mutation induced within the prostate cell AR by the cancer, and be a secondary event. The length of the prostate cancer cell AR CAG repeat will thus have no bearing on the predisposition to prostate cancer. As the aim of the present study was to analyse the correlation between CAG repeat length and this predisposition, the AR CAG repeat length was analysed in the DNA extracted from peripheral blood lymphocytes.

There was no association between the CAG repeat length in exon 1 of the human AR gene and the early onset of prostate cancer. The frequency distribution of CAG trinucleotide repeats was similar for both early- and late-onset prostate cancers (Fig. 1).

The association of microsatellites in the first exon of the human AR gene (the CAG and GGC repeats) with prostate cancer has been studied extensively [12,13,21–23]. Previous studies [13,14] reported an increase in the odds ratio for prostate cancer with decreasing CAG trinucleotide repeat length. In the present study there was no significant difference in CAG repeat lengths between the younger and older groups; these results are similar to those reported by Correa-Cerro *et al.* [24].

Circulating androgens stimulate the proliferation of most prostate adenocarcinomas and androgen withdrawal is standard therapy for most patients with advanced disease. Eventually the disease becomes androgen-independent and escapes therapeutic control, although the duration of response to androgen withdrawal varies widely. This loss of hormonal dependence is generally caused by the development of less differentiated tumour clones through accumulative genetic abnormalities occurring mainly during cell division [25]. Assuming that prostate cancer cells with short CAG repeats in the AR divide more rapidly, they would be more likely to give rise to androgen-independent cell clones early in the tumour progression. If this were so an association would be expected between short CAG repeats and high-grade prostate adenocarcinoma; such an association was reported by Giovanucci *et al.* [13] and a similar trend was reported by Bratt *et al.* [14]. However, the present results showed no such association or trend between short CAG repeats and high grade or stage of the tumour.

The incidence of prostate carcinoma and the length of the CAG repeat has been reported to vary with ethnicity [12,13]. Men of Asian ethnic origin have long CAG repeats and a low risk of prostate cancer, whereas African-Americans reportedly have short CAG repeats and a high risk of prostate cancer [12,13]; Caucasians are intermediate. Correa-Cerro *et al.* [24] studied the French-German population and found no relationship between the length of the CAG repeats and the stage or risk of prostate cancer;

this was contradictory to results in the USA [21–23]. Correa-Cerro *et al.* proposed that the mixed ethnicity of the American population was a cause of the relationship between short CAG repeats and high grade and early onset of prostate cancer. However, Bratt *et al.* [14] evaluated a population from Southern Sweden and reported that a short CAG repeat in the AR correlated with a lower age at diagnosis of sporadic and familial prostate cancer, but not with an increased risk of prostate cancer in general.

In the present patients from the West of Scotland, there was no correlation between the CAG repeat length and the age at diagnosis, or stage and grade of prostate cancer. These results, like those of Correa-Cerro *et al.* [24] cast doubt on the hypothesis that CAG repeat lengths have an influence on the susceptibility of the AR gene for prostate cancer. *In vitro* studies show that AR transcriptional activity is influenced by the CAG repeat length [26], but the significance of this remains unconfirmed in clinical practice.

The present study represents one of the largest cohorts of patients with prostate cancer in whom CAG repeats have been assessed; it seems that the length of the CAG repeat in the AR has little clinical significance in prostate cancer.

References

1 Demers RY, Swanson GM, Weiss LK, Kan TY. Increasing incidence of cancer of the prostate. *Arch Intern Med* 1994; **154**: 1211–6

2 Foster CS, Cornford P, Forsyth L, Djamgoz MBA, Ke Y. The cellular and molecular basis of prostate cancer. *Br J Urol* 1999; **83**: 171–94

3 Franks LM. Latent carcinoma of the prostate. *J Pathol Bacteriol* 1954; **68**: 603–16

4 Breslow N, Chan CW, Dhom G *et al.* Latent carcinoma of the prostate at autopsy in seven areas. *Int J Cancer* 1977; **20**: 680–8

5 Chisholm GD, Hedlund PO, Adolfsson J *et al.* The TNM system of 1992. Comments from the TNM working group. *Scand J Urol Nephrol* 1994; **162**: 107–14

6 Coffey DS. The molecular biology, endocrinology and physiology of the prostate and seminal vesicles. In Walsh PC, Retik AB, Stamey TA, Vaughan ED Jr eds, *Campbell's Urology*. Philadelphia: WB Saunders, 1992: 221–66

7 Bruchovsky N. Androgens and antiandrogens. In Holland JF, Frei E, Bast RC, Kufe DW, Morton DL, Weichselbaum RR eds, *Cancer Medicine*. 3rd edn. Philadelphia: Lea & Febiger, 1993: 884–96

8 Kyprianou N, English HF, Isaacs JT. Programmed cell death during regression of PC-82 human prostate cancer following androgen ablation. *Cancer Res* 1990; **50**: 3748–58

9 Chamberlain N, Driver E, Meisfeld R. The length and location of the CAG trinucleotide repeats in the androgen receptor N-terminal domain affect on transactivation function. *Nucl Acid Res* 1994; **22**: 3181–6

10 Mhatre AN, Trifiro MA, Kaufman M, Kazemi-Esfarjani P, Figlewicz D, Rouleau G. Reduced transcriptional regulatory competence of the androgen receptor in X-linked spinal and bulbar muscular atrophy. *Nat Genet* 1993; **5**: 184–8

11 Hakimi J, Rondinelli R, Schoenberg M, Banack E. Androgen receptor gene structure and function in prostate cancer. *World J Urol* 1996; **14**: 329–37

12 Irvine RYuM, Ross R, Coetzee G. The CAG and GGC microsatellites of the androgen receptor gene are in linkage disequilibrium in men with prostate cancer. *Cancer Res* 1995; **55**: 1937–40

13 Giovanucci E, Stampfer M, Krithivas K *et al.* The CAG repeat within the androgen receptor gene and its relationship to prostate cancer. *Proc Natl Acad Sci USA* 1997; **94**: 3320–3

14 Bratt O, Borg A, Kristoffersson U, Lundgren R, Zhang Q-X, Olsson H. CAG repeat length in the androgen receptor gene is related to age at diagnosis of prostate cancer and response to endocrine therapy, but not to prostate cancer risk. *Br J Cancer* 1999; **81**: 672–6

15 Sun S, Narod SA, Aprikian A, Ghadirian P, Labrie F. Androgen receptor and familial prostate cancer. *Nat Med* 1995; **1**: 848–9

16 Gleeson DF, Mellinger GT. The Veterans Administration Cooperative Urological Research Group. Prediction of prognosis for prostatic carcinoma by combined histological grading and clinical staging. *J Urol* 1974; **111**: 58–64

17 Beahrs OH, Myers MH. *American Joint Committee on Cancer. Manual for Staging of Cancer*. 2nd edn. Philadelphia: JB Lippincott, 1983: 159–64

18 Nanus DM, Mentle IR, Matzer RJ, Bander NH, Albino AP. Infrequent *ras* oncogene point mutations in renal cell carcinoma. *J Urol* 1990; **143**: 175–8

19 Sartor O, Zheng Q. Determination of CAG repeat length in the androgen receptor gene using frozen serum. *Urology* 1997; **49**: 301–4

20 Bartlett JM, Crilly A, White A, Madhok R. Modification of the GeneScan 2500 fluorescent dye standard for accurate product sizing. *Mol Biotechnol* 1999; **13**: 185–9

21 Ingles SA, Ross RKYuMC, Irvine R, La Pera G, Haile R, Coetzee G. Association of prostate cancer risk with genetic polymorphisms in Vit D receptor and androgen receptor. *J Natl Cancer Inst* 1997; **89**: 166–70

22 Hardy DO, Scher HI, Bogenreider T *et al.* Androgen receptor CAG repeat lengths in prostate cancer. correlation with age of onset. *J Clin Endocrinol Metab* 1996; **81**: 400–405

23 Stanford JL, Just JJ, Gibbs M *et al.* Polymorphic repeats in the androgen receptor gene. molecular markers of prostate cancer risk. *Cancer Res* 1997; **57**: 1194–8

24 Correa-Cerro L, Wohr G, Huassler J *et al.* (CAG) nCAA and GGN repeats in the human androgen receptor gene are not associated with prostate cancer in a French-German population. *Eur J Human Genet* 1999; **7**: 357–62

25 Pinker-Shaeffer C, Partin A, Isaacs W, Coffey D, Isaacs J. Molecular and cellular changes associated with the acquisition of metastatic ability by prostate cancer cells. *Prostate* 1994; **25**: 249–65

26 Kazemi-Esfarjani P, Trifiro MA, Pinsky L. Evidence for a repressive function of the long polyglutamine tract in the human androgen receptor: possible pathogenetic relevance for the (CAG) n-expanded heamopathies. *Human Mol Genet* 1995; **4**: 523–7

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Abbreviations: **AR**, androgen receptor.