Variation in CAG and GGN repeat lengths and CAG/GGN haplotype in androgen receptor gene polymorphism and prostate carcinoma in Nigerians

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ABSTRACT

Prostate cancer has become the most common cancer in Nigerian men. The growth of the prostate gland depends on circulating androgens and intracellular steroid signalling pathways. The effects of androgens are mediated through the androgen receptor (AR), a nuclear transcription factor encoded by the *AR* gene. The common polymorphisms, CAG and GGN repeats, in exon 1 of this gene have been implicated as possible risk factors. Thus far, existing supporting data are scanty and none are from sub-Saharan African populations. Therefore, this study investigates the possible association between *AR* polymorphism repeat length (CAG and GGN) and prostate cancer in Nigerians. A total of 261 subjects (70 with prostate cancer, 68 with benign prostate hyperplasia [BPH], 123 age-matched apparently normal subjects as controls) were studied. CAG and GGN repeats length were determined by fragment length analysis using GeneScan. The CAG repeat length in prostate cancer and in BPH compared to the controls was significantly different ($P<0.05$) with reduce length of CAG repeats showing a significant odds ratio (OR) in both cases. However, this was not observed in GGN repeat length, which showed no significant difference between cases and controls ($P>0.05$). CAG and GGN haplotype variation showed no significant difference between cases and controls ($P>0.05$), except that the haplotypes CAG≥21 and GGN≤21 were more common in the control group. The results of this study, the first from sub-Saharan Africa, supports the hypothesis that reduced CAG repeat length is a risk factor for prostate cancer, and also suggests an association with BPH.

KEY WORDS: Haplotypes.
Prostatic neoplasms.
Receptors, androgen.

Introduction

Prostate cancer is the most common cancer in Nigerian men and constitutes 11% of all male cancers.[1] Despite an expanding body of epidemiological data, the aetiology of prostate cancer remains poorly understood. However, evidence supports the involvement of genetic and environmental factors, which may also contribute to the ethnic differences in incidence rates.[2]

The growth of the prostate gland depends on circulating androgens and intracellular steroid signalling pathways. The effects of androgens are mediated through the androgen receptor (AR), a ligand-activated nuclear transcription factor encoded by the *AR* gene, located on the X chromosome (Xq11–12). The *AR* gene comprises eight exons, spanning more than 90 kb of the genomic DNA. Exon 1 of the *AR* gene contains polymorphic CAG and GGC repeats encoding polyglutamine and polyglycine tracts, respectively. Androgens bind to the AR, stimulating transcription of a cascade of androgen-responsive gene products (e.g., prostate-specific antigen [PSA]) and genes involved in cell cycle control.[3]

The incidence of clinical prostate cancer differs substantially between ethnic groups, and is highest in Western countries, especially among African American men, and lowest in developing countries.[4] However, little is known about why certain populations are more susceptible. It has been suggested that varying levels of androgens across ethnic groups may be responsible for these differences.[4] The ethnic variation in the GGN and CAG microsatellites of the *AR* gene suggests that they have a role in the substantial racial difference in prostate cancer risk.[2] *In vitro* investigations suggest that variation in (CAG)n affects AR transactivation.[5]

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Studies of CAG repeat variation in prostate cancer risk are inconsistent. In India, a study conducted on a north Indian population showed significant association,[6] and similar observations were recorded in China.[7] In addition, several epidemiological studies have shown that shorter CAG and GGN repeat length confers a higher risk of prostate cancer.[8–10] However, much of the published data supporting a relationship between *AR* polymorphisms and prostate cancer come from white population, and presently there are no data from Nigeria.

To assess the importance of *AR* polymorphisms in prostate cancer, this prospective case-control study examines the polymorphic length of CAG and GGN repeats in relation to

Table 1. Group statistics for clinical parameters evaluated in patients and controls.

	PCa (*n*=70)	BPH (*n*=68)	Controls (*n*=123)	F	*P* values
Age (years)	63.5±8.38	62.15±5.57	62.3±5.87	2.15	0.123
PSA	89.83±127.35	14.86±17.07	1.57±1.16	40.83	0.000
CAG	19.37±2.96	19.29±3.24	21.04±2.6	8.10	0.000
GGN	21.18±2.53	20.86±2.44	20.22±3.08	1.77	0.173

Values expressed as mean±standard deviation.

Statistical analysis by ANOVA.

risk of prostate cancer and benign prostatic hyperplasia (BPH) in Nigeria.

Materials and methods

Participants were recruited with informed consent from the Cancer Screening Unit (CSU), University College Hospital (UCH), Ibadan, Nigeria, and comprised a total of 261 subjects (70 prostate cancer patients, 68 with benign prostate hyperplasia, and 123 age-matched apparently normal subjects as controls). Patients had no recent hormone therapy and/or radiation therapy. The study received ethics approval from the Oyo State Ministry of Health, Nigeria.

Sample collection

Blood samples were drawn from the antecubital vein into commercial tubes containing EDTA and also into plain centrifuge tubes. The plain samples were allowed to clot and then centrifuged at 3000 *xg* for 15 min to obtain serum, which was used for PSA determination. The EDTA-anticoagulated blood was used for DNA extraction.

Prostate-specific antigen assay

Assessment of PSA in serum was performed using an electrochemiluminescence immunoassay (Roche Diagnostics) in combination with a Roche/Hitachi Modular Analytics analyser.

Table 2. Odds ratios (OR) and 95% confidence interval (CI) for prostate cancer in relation to the number of CAG and GGN repeats in the *AR* gene.

Repeats		PCa *n*=70	BPH *n*=68	Controls *n*=123	OR		95% CI
CAG							
Median	>21	30 (43%)	28 (41%)	73 (59%)	a=0.51 b=0.48	*P*=0.03 *P*=0.02	0.29–0.93 0.26–0.87
	≤19	32 (46%)	30 (44%)	23 (19%)	a=3.66 b=3.43	*P*=0.00 *P*=0.00	1.91–7.01 1.78–6.61
Tertile	≥22	20 (29%)	20 (29%)	56 (46%)	a=0.48 b=0.50	*P*=0.02 *P*=0.03	0.26–0.89 0.27–0.93
	19–21	22 (31%)	16 (24%)	53 (43%)	a=0.61 b=0.41	*P*=0.13 *P*=0.01	0.33–1.12 0.21–0.79
	≤18	28 (40%)	26 (38%)	14 (11%)	a=5.19 b=5.45	*P*=0.00 *P*=0.00	2.51–10.72 2.63–11.23
GGN							
Median	>21	38 (54%)	32 (47%)	52 (42%)	a=1.62 b=1.21	*P*=0.13 *P*=0.55	0.90–2.92 0.67–2.20
	≤21	32 (46%)	36 (53%)	71 (58%)	a= 0.62 b= 0.82	*P*=0.13 *P*=0.55	0.34–1.11 0.46–1.49
CAG≥21 GGN>21		10	8	36	a=0.40 b=0.32	*P*=0.02 *P*=0.01	0.19–0.86 0.14–0.73
CAG≥21 GGN≤21		4	4	8	a=0.87 b=0.90	*P*=1.00 *P*=1.00	0.27–2.84 0.28–2.93
CAG≤9 GGN>21		18	20	24	a=1.43 b=1.72	*P*=0.37 *P*=0.15	0.72–2.85 0.87–3.40
CAG≤19 GGN≤21		6	10	11	a=0.96 b=1.76	*P*=1.00 *P*=0.24	0.35–2.62 0.72–4.29

a: odds ratio or risk of having prostate cancer

b: odds ratio or risk of having BPH

Genetic analysis

Genomic DNA was extracted from EDTA-anticoagulated whole blood using the FlexiGene DNA kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. Primers were designed to amplify exon 1 of *AR* gene.

CAG and GGN repeat lengths

Primers were labelled with [γ-^{33}P] ATP using T4-polynucleotide kinase (Eurogentec SA, Belgium). Approximately 10–50 ng genomic DNA was subjected to 30 cycles of PCR amplification. The CAG primers were 5′-TCC AGA ATC TGT TCC AGA GCG TGC-3′ and 5′-GCT GTG AAG GTT GCT GTT CCT CAT-3′. The PCR amplification was performed as follows: 94°C for 45 sec, 59°C for 30 sec and 72°C for 1 min. The GGN primers were 5′-TCC TTG CAC ACT CTC TTC AC-3′ and 5′-GGC AGG GTA CCA CAC ACT AGG T-3′. The PCR amplification was performed as follows: 95°C for 45 sec, 61°C for 45 sec and 72°C for 90 sec. Products were separated by electrophoresis in 2% polyacrylamide formamide gel, followed by autoradiography. Products, for which CAG and GGN repeat lengths were identified by sequencing, were used as a reference.

Statistical analysis

The χ^2 test was used to compare the difference in allele frequency between patients and controls. Values are expressed as mean ± SD. Student's *t*-test was used to compare means between groups using SPSS 14.0 (SPSS. Chicago, USA), after checking for normal distribution.

Results

The mean ages of the prostate cancer and BPH patients and the healthy controls were 63.5, 62.2 and 62.3 years, respectively. Mean serum PSA levels measured at the time of diagnosis were 89.83 ng/mL, 14.86 ng/mL and 1.57 ng/mL in prostate cancer and BPH patients and in controls, respectively. Selected characteristics of the cases and controls are presented in Table 1.

The number of CAG repeats among cases and healthy controls were 11–24 and 11–30, respectively, with a median of 19 CAG in both patient groups, but 21 CAG in the healthy control group ($P<0.05$). The number of GGN repeats were 17–28 in prostate cancer patients and 14–24 in BPH, with a median of 22 and 21 repeats, respectively. Numbers in the control group were 14–28, with a median of 21 ($P<0.05$) (Figs. 1 and 2).

Some 59% of the controls had CAG repeats ≥21 while 19% had CAG repeats ≤19. In contrast, 48% and 44% of prostate cancer and PBH has CAG repeats ≤19. However, this significant pattern was not observed with GGN repeats, which showed no significant difference in percentage distribution between cases and controls (Figs. 3 and 4).

Possible CAG and GGN haplotype variation showed no significant difference between cases and controls ($P>0.05$), except that the haplotypes CAG≥21 and GGN≤21 were more common in the control group. (Table 2). Although no significant correlation was seen between short CAG alleles and PSA level ($P>0.05$), a significant difference in median

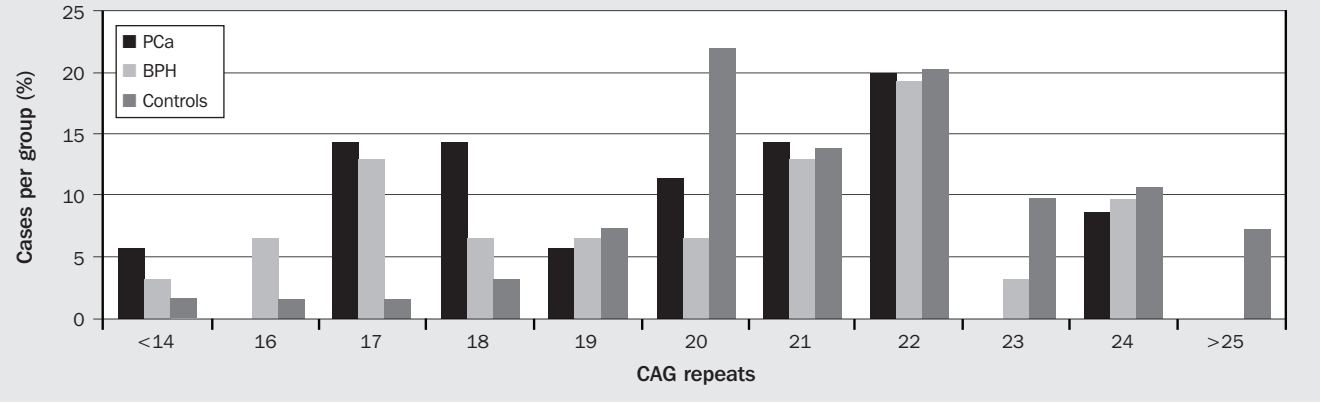

Fig. 1. Distribution of CAG repeats in the *AR* gene in cases and controls.

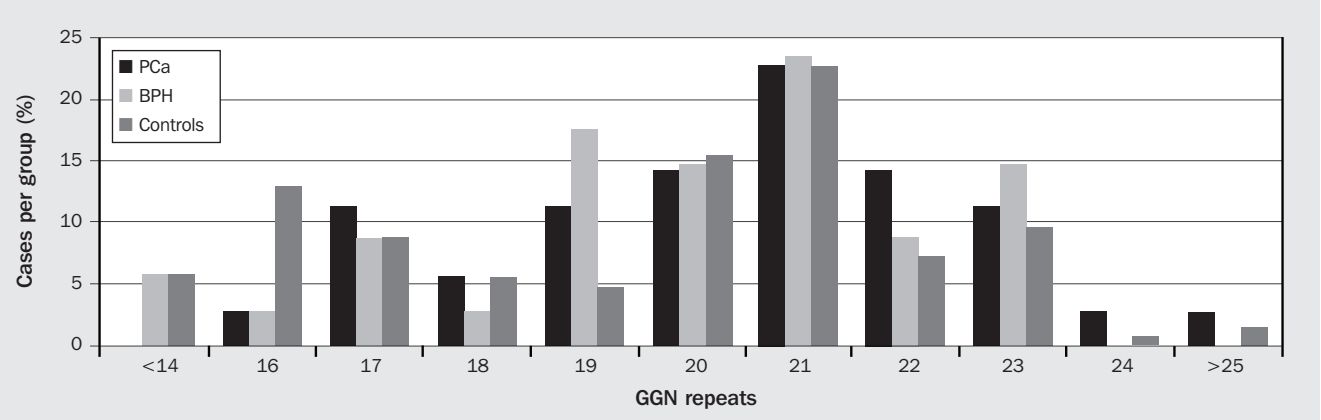

Fig. 2. Distribution of GGN repeats in the *AR* gene in cases and controls.

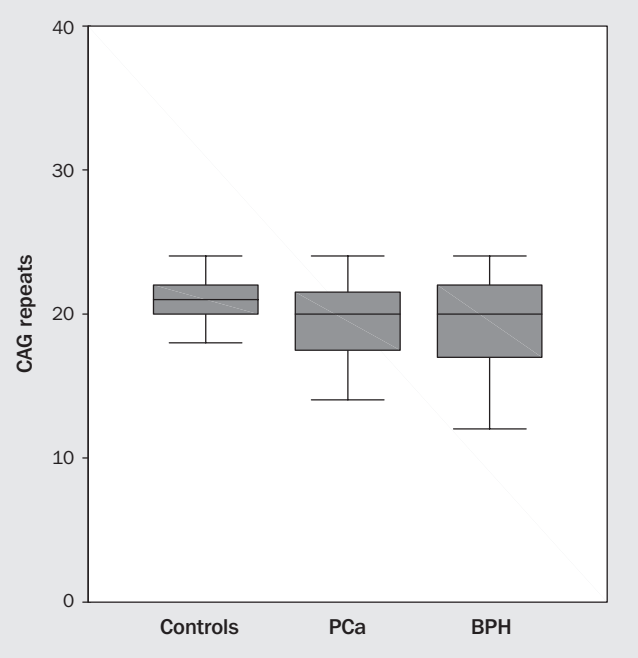

Fig. 3. Plot box showing distribution of CAG repeats among PCa and BPH cases and controls.

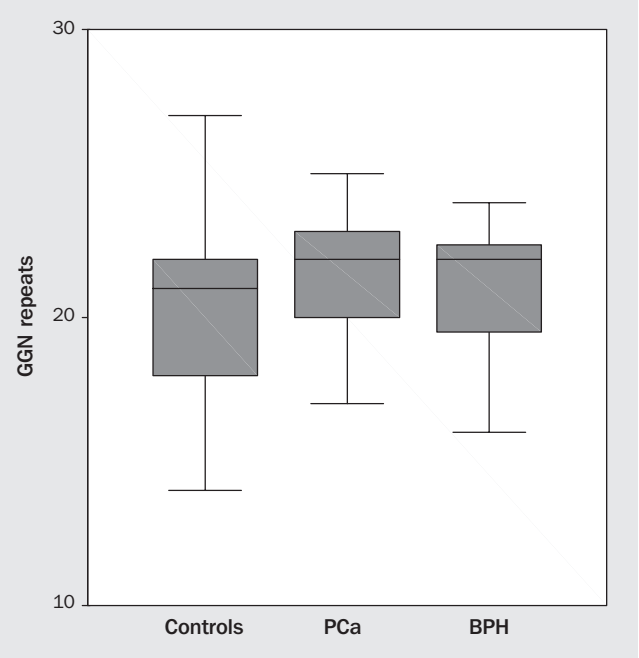

Fig. 4. Plot box showing distribution of GGN repeats among PCa and BPH cases and controls.

CAG repeats in each group correlated with PSA level ($P<0.05$) (Fig. 5).

Discussion

Results from this study, the first on a Nigerian low-risk population,[4,11] suggest that a shorter CAG repeat length is associated with an increased risk of clinically significant prostate cancer. Longer GGN repeat length also appears to reduce the risk of prostate cancer, but the influence did not reach statistical significance. These findings agree with previous studies which showed that short CAG repeat lengths in the *AR* gene predispose to prostate cancer.[2,4,6–10,12–15] However, this differs from the results of others, especially in French-German populations that reported no such association.[16,17] The association between a short CAG repeat and prostate cancer is due to enhanced transactivation activity[5] or increased messenger RNA (mRNA) levels[18] observed in *in vitro* experiments using *AR* genes with fewer CAG repeats. Also, in contrast to a previous study by Bousema *et al*.,[19] which reported no association between CAG repeat polymorphism in the *AR* gene and risk of BPH, the present study found CAG repeat length to be significantly different in BPH patients compared to controls. These results suggest the possibility that risk of malignancy may be higher in BPH patients compared to controls, a school of thought supported by other previous studies.[20–23] Studies have also found increased AR protein expression levels in BPH and other diseased prostatic tissue from men of African descent;[24] however, further study is required to confirm this association.

Interestingly, no significant association was seen between short CAG alleles and PSA level, and is in accord with the work of Mittal *et al*.[25] This observation highlights the fact that PSA is not a sensitive indicator for prostate cancer. It is prostate specific, but not prostate cancer specific, and also lacks the sensitivity to detect a large proportion of early-stage tumours, as >15% of men with a normal serum PSA level have biopsy-proven prostate cancer.[26] Serum PSA level can be altered by medication, prostatitis and urological manipulation, as well as BPH, as indicated by the results of the present study. However, a significant difference in median CAG repeat length with PSA level was seen (Fig. 5).

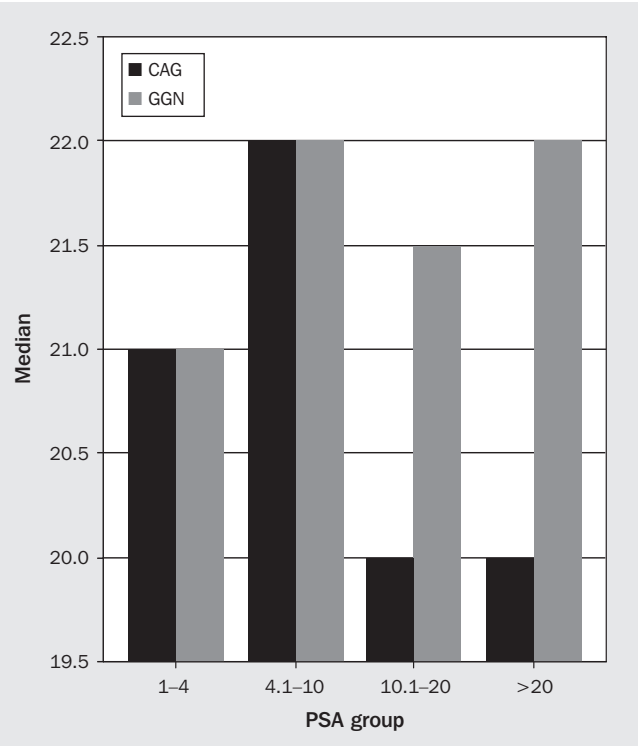

Fig. 5. Distribution of CAG and GGN repeats among different groups of PSA levels in all subjects. 1–4: normal; 4.1–10: mildly increased; 10.1–20: 25% probability of metastasis; >20: highly suspicious.

This supports findings that BPH patients with *AR* CAG instability had a 12-fold increased risk for development of prostate carcinoma.[17]

In contrast to the observations of Vijayalakshmi *et al.*,[2] which showed that specific GGN/CAG haplotypes (CAG≤19/GGN≤21 and CAG≤19/GGN>21) of the *AR* gene increase the risk of prostate cancer, and thus could serve as a susceptibility marker for prostate cancer, no such association was seen in the present study. However, it did show a lower incidence of prostate cancer and BPH associated with the CAG/GGN haplotype CAG≥21/GGN≤21.

In conclusion, this study reports the pattern of CAG and GGN repeat polymorphisms in prostate cancer and BPH in Nigerian males. While the data support previous findings of a direct relationship between CAG and prostate cancer, it also suggests some association with BPH. Although no direct relationship between PSA level and *AR* repeat polymorphisms was observed, the results demonstrated some CAG instability as PSA level increases. These results provide potential tools to assist prediction strategies in this important disease. Furthermore, association of the haplotype CAG≥21/GGN≤21 with normal controls warrants further investigation. □

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