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Tandem CAG Repeats of the Androgen Receptor Gene and Prostate Cancer Risk in Black and White Men

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The most common malignancy in men worldwide is cancer of the prostate. Androgens play a direct role in normal and malignant growth of prostate cells via the androgen receptor (AR). This study analyzed the polymorphic CAG repeat sequence in exon 1 of the AR gene to determine if the number of repeats might be an indicator of prostate cancer risk or aggressive disease. DNA was extracted from blood samples of 20 black and 20 white men with well-documented prostate cancer and 40 healthy controls (20 blacks and 20 whites). PCR amplification was followed by gel electrophoresis and DNA sequencing. This region normally contains between 9 and 29 repeats. Patients and controls both had minor variations in the number of repeats, which ranged from 13 to 27 with 21 being the most frequent allele. Black controls and patients both had a mean of 20 ± 3 repeats; in whites the mean was significantly lower in patients than controls (21 ± 2 versus 23 ± 2; $p = 0.004$). Combined black and white patients also had a lower number than the combined group of controls (20 ± 3 versus 22 ± 3; $p = 0.02$). Similarly, black and white patients with aggressive disease had a lower number than patients whose disease was more slowly progressive (19 ± 2 versus 22 ± 3; $p = 0.02$). We conclude that the small differences in the number of CAG repeats in both black and white patients do not appear to be a strong indicator of risk or aggressive disease but that this size polymorphism may be one of many genetic and environmental risk factors involved in prostate cancer.

Key Words: Androgen receptor gene; prostate cancer risk.

Introduction

The most common male malignancy worldwide is cancer of the prostate *(1,2)*. In South Africa, 1 in 31 men has a lifetime risk of developing the disease *(3)*. The mortality rate is rising so rapidly that it has become the most common cancer among South African white men and the second most common cancer in black men after cancer of the esophagus *(3)*. A striking feature of prostate cancer epidemiology is the marked variation among different racial–ethnic groups. Prevalence and mortality rates are higher in black Americans and Scandinavians and lower in Orientals living in Asia *(4)*.

Previous studies have suggested that androgens are involved in prostate cancer etiology *(5,6)*. Androgens play a direct role in the development, growth, and differentiation of normal and malignant prostate cells via the androgen receptor (AR), which binds dihydrotestosterone. An important function of the transcript of the AR gene is to stimulate the expression of other androgen-responsive genes, including prostate-specific antigen (PSA). This regulatory activity resides in the N-terminal domain of the protein that is encoded in exon 1 of the AR gene *(7)*. This exon contains a highly polymorphic microsatellite, comprising a trinucleotide CAG repeat sequence *(8)*. It has been hypothesized that the transcriptional regulatory activity of the AR correlates with the length of this CAG repeat sequence and that progressive elimination of repeats increases the level of receptor transactivation activity *(9)*.

The biological consequences of variations in the length of this segment are not clear. In some instances, however, alterations in its size have been associated with changes in function. A substantial increase in the number of repeats to 40 or more causes X-linked spinal and bulbar muscular atrophy, described as Kennedy's disease *(10)*, which is associated with features of androgen resistance. A truncation of the CAG repeat region to less than the common number of 20–22 residues may be related to prostate cancer *(11)*.

Interethnic differences in the average length of the CAG repeat sequence have been found in black Americans, whites, and Asians, that show an inverse relationship to their prevalence rates mentioned above *(12)*. Based on this association, the aim of the study was to analyze the CAG repeat region in black and white men to establish whether or not the number of repeats is an indicator of risk for prostate cancer or a marker of aggressive disease.

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Patients and Methods

Patients

Blood samples for DNA studies were taken from 20 unrelated black and 20 unrelated white men with previously documented prostate cancer (PSA median = 38 μg/L, range = 2–4130 μg/L and composite Gleason score median = 8, range = 5–9). Aggressive disease, defined as poorly differentiated tumors with a Gleason score of 8–10, and/or regional or metastatic (stage C or D) dissemination, was diagnosed in six black and six white patients. The majority of patients had received either a medical orchidectomy with long-acting gonadotropin-releasing hormone (GnRH) agonists or a surgical orchidectomy; none had undergone prior radiotherapy.

The control group consisted of 40 apparently healthy men (20 black and 20 white) with PSA levels in the normal range (0.0–4.0 μg/L). White patients and controls were obtained from both Israel and South Africa (Jerusalem and Johannesburg), while black patients and controls were recruited from South Africa. All subjects gave their informed consent to participate in the study, which was approved by the Committees for Research on Human Subjects of the University of the Witwatersrand and the Shaare Zedek Medical Centre.

Polymerase Chain Reaction (PCR)

The CAG repeat sequence of the AR gene was amplified by PCR using genomic DNA from each subject as template. The nucleotide sequences of the 5' and 3' PCR primers were 5'-GCC TGT TGA ACT CTT CTG AGC-3' and 5'-GCT GTG AAG GTT GCT GTT CCT C-3', respectively. Reactions were performed in a final volume of 100 μL containing 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.4), 1.0 mmol/L $MgCl_2$, 0.01% gelatin, 0.2 mmol/L of each deoxyNTP, 50 pmol of each primer, and 8 U AmpliTaq DNA polymerase (PE Applied Biosystems). Reaction mixtures were denatured for 5 min at 95ºC, followed by 35 cycles of amplification (1 min at 95ºC, 2 min at 65ºC, and 1.5 min at 72ºC). The final extension step lasted 8.5 min. Aliquots (25 μL) of the amplified products were separated on a 3% high-resolution agarose gel. The remainder of the PCR products were purified using a QIA Quick Spin Purification Kit (Qiagen GmbH, Hilden, Germany).

Direct Sequencing of PCR Products

Bidirectional sequencing was performed with a PE Applied Biosystems Big Dye™ Terminator Cycle Sequencing Ready Reaction Kit, followed by analysis on an Applied Biosystems Prism® 310 Automated Genetic Analyser. Five different CAG alleles were sequenced containing 13, 17, 18, 21, and 27 CAG repeats. The number of CAG repeats in the other samples was calculated by comparing the size of PCR products of unknown alleles with the PCR products from individuals with a sequenced allele.

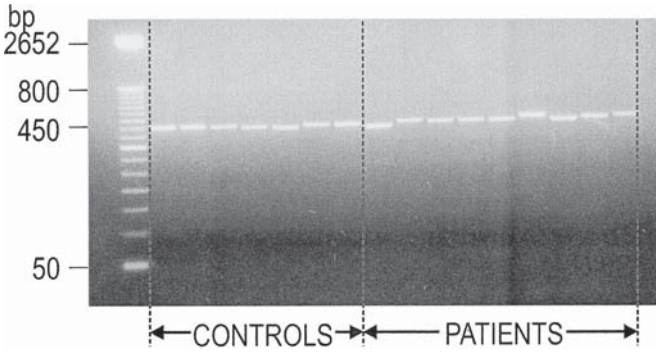

Fig. 1. Agarose gel electrophoresis of PCR products obtained by amplification of the androgen receptor CAG repeat region in DNA samples from control subjects (lanes 2–8) and patients with prostate cancer (lanes 9–17).

Measurement of Prostate-Specific Antigen (PSA)

PSA levels in serum were measured by immunoassays using kits supplied by Chiron Diagnostics and BYK, Sang Diagnostica.

Statistical Analysis

Data were analyzed using the two-tailed unpaired *t* test (parametric) or Wilcoxon signed rank test (nonparametric) as appropriate. Pearson's coefficient of correlation (*r*) was computed between ages of subjects and numbers of CAG repeats. A value of $p < 0.05$ was considered significant. Results are expressed as mean ± SD.

Results

PCR amplification of the DNA segment containing the CAG repeat region in control subjects and patients showed bands representing fragments that were all within the expected range, approx 400–450 basepairs (Fig. 1). The CAG repeat region typically contains between 9 and 29 repeats *(13)*. Sequencing analysis showed that controls and patients had small variations in the number of repeats, which ranged from 13 to 27, with 21 being the most frequent allele.

The distribution of CAG repeats in black and white controls and patients is shown in Fig. 2. In the black group, controls and patients both had a mean number of 20 ± 3 repeats; however, in the white cohort it was significantly lower in the patients than in the controls (21 ± 2 versus 23 ± 2; $p = 0.004$). The combined black and white patients also had a lower mean number of repeats than the combined group of controls (20 ± 3 versus 22 ± 3; $p = 0.02$). Similarly, black and white patients with aggressive disease (Gleason score 8–10 and/or metastases) had a lower number of repeats than patients whose disease appeared to be more slowly progressive (19 ± 2 versus 22 ± 3; $p = 0.02$).

The majority of patients were age 60 or older when studied (blacks = 68 ± 11 yr; whites = 76 ± 6 yr), and there was no correlation between their ages and CAG repeat num-

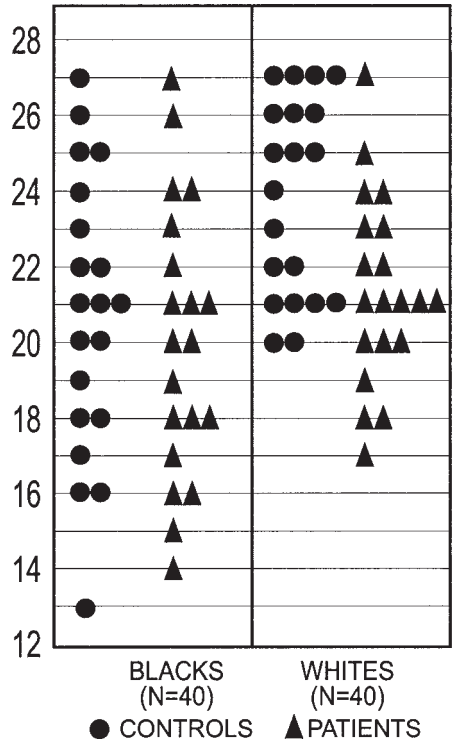

Fig. 2. Allele frequencies of the CAG repeat sequence at the androgen receptor locus in black and white patients and controls.

bers. However, there was a significant correlation between these variables in four black patients who were less than 60 yr and had a mean repeat number of 19 ($r = 0.99$; $p = 0.01$).

Discussion

There has been much interest in the role of the AR gene, particularly the CAG repeat region, in the pathogenesis of prostate cancer. Some researchers *(5)*, but not all *(14)*, have concluded that there is an inverse relationship between smaller CAG size and greater transcriptional activity. It is possible, therefore, that shorter CAG alleles cause more rapid growth of prostate cells, which, in turn, increases prostate cancer risk.

In the present study, we analyzed the CAG repeat sequence in black and white men to determine if variability in the number of repeats is an indicator of risk for prostate cancer or a marker of aggressive disease. The frequency profile shifted slightly, but significantly, toward a lower number of repeats and therefore shorter alleles, in white patients compared to white controls and the combined black and white patient group. This finding confirms a report by Irvine et al. *(15)* who also found that more white patients had short repeats, but differs from a study by Stanford et al. *(13)* where no significant difference between white patients and controls was found.

Previous studies have demonstrated correlations between a younger age (less than 60 yr) at diagnosis and more aggressive disease *(16)*, as well as younger age and short CAG repeats *(17)*. In a small subset of our younger black patients, short repeats were correlated with current age. The large majority of black and white patients were age 60 or older, and their ages were not related to the numbers of CAG repeats. However, analysis according to histological tumor grade (Gleason score 8–10 and/or metastases) showed that, in the presence of underlying prostate cancer, shorter CAG repeats were associated with more aggressive disease in both ethnic groups.

Epidemiological observations suggest that the etiology of prostate cancer is multifactorial, with a combination of genetic and environmental risk factors influencing its development *(18)*. There is some evidence for inherited and genetic components and several mutations have been reported in genes such as *steroid 5α-reductase*, *ras*, KAI 1, *C-erbB*, *E-caderin*, *p53* as well as the *AR* gene itself *(19–21)*. Up to 10% of men with prostate cancer have a family history of the disease *(22)*. Diet is one of the most important lifestyle factors, and a high consumption of saturated fat is linked to increased risk *(23)*. The influence of the environment is further suggested by the increased prevalence of prostate cancer in second generation Chinese and Japanese Americans *(24)*. This phenomenon might also be happening in South Africa as a result of rapid urbanization and the adoption of a modern Western lifestyle, particularly among the black population *(25)*.

Our study had certain limitations in that the number of subjects was relatively small, they had a narrow age distribution, and there was incomplete follow-up data on response to medical therapy. Moreover, the white subjects consisted of individuals from Israel and South Africa, whose origins are derived from a wide variety of European and Sephardi communities either by historical or more recent immigration. Although a larger multinational study would have yielded more definitive information, these reservations do not detract from the overall interpretation of our findings.

We conclude that the small variations that were found in the number of CAG repeats in both black and white patients do not appear to be a strong indicator of risk, but that this size polymorphism may be one of several genetic and environmental risk factors involved in the pathogenesis or aggressiveness of prostate cancer.

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