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South Indian men with reduced CAG repeat length in the androgen receptor gene have an increased risk of prostate cancer

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Abstract The androgen receptor (AR) gene possesses polymorphic CAG tandem repeats and the repeat length has been inversely related to the risk of prostate cancer (PCa). The distinct ethnic variation in the CAG repeat length may be correlated to differences in PCa risk in different populations. To evaluate the CAG repeat length in the AR gene and the implications for PCa, we screened 87 PCa patients and 120 control subjects from South India. The mean CAG repeat length in PCa patients was significantly smaller than that of controls (17.0 vs 20.7; $P < 0.001$). Men with ≤ 19 CAG repeats had a significantly increased risk of cancer compared to those with > 19 CAG repeats (age-adjusted OR = 7.01; 95% CI = 3.52–13.94; $P < 0.001$). However, no significant association was observed between CAG repeats and age of onset or prostate-specific antigen levels. Although there was a trend towards shorter CAG repeat length in high grades of cancer, it was not significant ($P = 0.085$). Thus, our results suggest an association between short CAG repeats in the AR gene and PCa risk in South Indian men. Further, we propose that CAG repeats could be used as a prognostic marker for PCa diagnosis.

Keywords Androgen receptor · CAG repeats · Prostate cancer · Genotype · STR

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Introduction

Prostate cancer (PCa), one of the most common malignancies in men, exhibits obscure etiology. The growth of the prostate gland is dependent on circulating androgens and intracellular steroid signalling pathways. The effects of androgen are mediated through the androgen receptor (AR), a ligand-activated nuclear transcription factor. Androgens bind to the AR, stimulating transcription of a cascade of androgen-responsive genes such as prostate-specific antigen (PSA) and genes involved in cell-cycle control (Lu et al. 1997). Thus, AR transactivation is important for the normal growth and function of the prostate. The transactivation domain encoded by exon 1 of the AR gene (Xq11-12) contains polymorphic CAG and GGC repeats encoding polyglutamine and polyglycine tracts, respectively. The effect of GGC repeat variation on AR activity and its association with prostate cancer risk are unclear. The number of CAG repeats varies greatly among populations, ranging from 8 to 31 with an average of about 20 repeats (Edwards et al. 1992). Irvine et al. (1995) reported the mean CAG repeat number to be smallest in African Americans, intermediate in Caucasians and largest in Asians, populations having high, intermediate and low incidence of PCa, respectively. In vitro investigations suggest that variation in (CAG) n affects AR transactivation (Chamberlain et al. 1994).

Studies on CAG repeat variation in PCa risk have been inconsistent. In India, the one study conducted on the North Indian population showed significant association (Mishra et al. 2005); however, there have been no studies on South Indian men to date. Since India is known for its unique population structure, having about 5,000 endogamous populations, one would expect CAG repeat length variation among South Indians to be different. Therefore, we have attempted to analyse the association of CAG repeat number in the AR gene of PCa patients as well as control men from the same

ethnic background, and to understand whether repeat length is associated with the age of onset, PSA levels, and/or cancer progression.

Materials and methods

Subjects

Our study comprised 87 histologically confirmed PCa patients and 120 age-matched male control subjects from South India, with mean ages of 67.5 and 66.5 years, respectively. The controls comprised 80 healthy, unrelated individuals with normal serum PSA levels (≤ 4 ng/ml), digital rectal examination showing no abnormality, with no history of cancer, and 40 subjects with benign prostatic hyperplasia (BPH). The study was approved by the Institutional Medical and Ethics Committee.

Genotyping

Blood samples were collected from the subjects with informed written consent. Genomic DNA isolated from blood was genotyped for exon 1 of the AR gene by PCR followed by gene scan analysis on an ABI 3730 Genetic Analyzer at the Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India, with conditions as described in Mishra et al. (2005).

Statistical analysis

The mean number of CAG repeats incases in different groups of Gleason Score (GS), age of onset, and PSA, and the distribution of repeats in cases and controls, were compared by Student's unpaired 't' test. Odds ratio (OR) for the repeats among cases and controls was calculated by adjusting for subject age using a binary logistic regression method. A χ^2 test was performed to compare two or more proportions between cases and controls. Stepwise multiple logistic regression analysis was carried out with CAG repeat number as dependent variable, and age of onset, PSA and tumour grades as co-variables. All tests were two-sided with 5% level of significance and were performed using SPSS (version 13).

Fig. 1 Distribution of CAG repeats in the androgen receptor (AR) gene among prostate cancer (PCa) cases and controls

Results

The CAG repeat distribution in PCa and controls is shown in Fig. 1. The mean number of CAG repeats in cases, healthy controls and BPH controls was 17, 20.7 and 21.3, respectively. The mean number of CAG repeats in PCa patients was significantly smaller than that of both the BPH and healthy controls ($P < 0.001$). Among the control group, there was no significant difference in the mean CAG repeat length between BPH and healthy controls (21.3 vs 20.5). The study subjects were dichotomised based on mean CAG repeats in cases and controls (19 repeats). Men with ≤ 19 CAG repeats had a 5-fold increased risk for PCa compared to those with > 19 repeats (crude OR = 5.90 at 95% CI = 3.2–11.2; $P < 0.001$). However, the age-adjusted OR revealed a 7-fold increased risk in men with ≤ 19 CAG repeats compared to those with > 19 repeats (adjusted OR = 7.01 at 95% CI = 3.5–13.9; $P < 0.001$) (Table 1).

With respect to tumour grade, patients with well and moderately differentiated tumour were classified as low grade (GS < 7) and those with poorly differentiated tumour as high grade (GS ≥ 7). Although a trend towards short mean CAG repeat length with high grade of cancer was observed, it was non-significant ($P = 0.085$). With respect to age at onset, subjects were stratified by age into four groups based on quartiles (≤ 62 , 63–66, 67–72, > 72), and into two groups with regard to PSA levels, with mean PSA value as the cut-off. The mean CAG

Table 1 Distribution of CAG repeats in the androgen receptor (AR) gene among prostate cancer (PCa) cases and controls. OR Odds ratio, Adj OR adjusted odds ratio, CI confidence interval

No. of CAG repeats	PCa cases (n = 87)	Controls (n = 120)	Crude OR	95% CI	Adj OR ^a	95% CI
> 19	20	77	1.00		1.0	
≤ 19	67	43	5.90*	3.20–11.20	7.01*	3.52–13.94

* $P < 0.001$

^aAdjusted for age

Table 2 Comparison of CAG repeats of PCa cases as a variable with age of onset, grade and serum prostate-specific antigen (PSA) levels at diagnosis. GS Gleason score

Variable	No. of cases (n)	No. of CAG repeats (mean $\pm$ SD ^a)	P value	Distribution of CAG repeats within each prognostic factor		P value
				No. of cases with ≤ 19 CAG repeats	No. of cases with > 19 CAG repeats	
Grade						
GS < 7 (low grade)	48	17.6 $\pm$ 2.8	0.085	36	12	NS ^b
GS ≥ 7 (high grade)	39	16.3 $\pm$ 3.7		31	8	
Age						
≤ 62	22	17.3 $\pm$ 2.9	NS	18	4	NS
63–66	24	17.4 $\pm$ 3.1		18	6	
67–72	24	17.0 $\pm$ 3.2		18	6	
> 72	17	16.1 $\pm$ 4.1		13	4	
PSA level						
≤ 48	54	16.9 $\pm$ 3.5	NS	41	13	NS
> 48	33	17.2 $\pm$ 3.1		26	7	

^aStandard deviation^bNon-significant

repeat lengths within each of the age groups and PSA groups revealed no significant difference. Stepwise multiple logistic regression analysis of the CAG repeat distribution on the age of onset, grade, and PSA level revealed no significant association with any of the variables (Table 2).

Discussion

Short CAG repeat length in the AR gene has been hypothesised to predispose to PCa (Coetzee and Ross 1994). Our study revealed that men with ≤ 19 CAG repeats have a 7-fold increased risk of prostate cancer compared to those with > 19 CAG repeats; no significant association was seen between short CAG alleles and age, PSA levels, or high-grade cancer. The association between a short CAG repeat and PCa might be due to the enhanced transactivation activity (Chamberlain et al. 1994) or increased mRNA levels (Choong et al. 1996) observed in in vitro experiments using AR genes with fewer CAG repeats. CAG repeat length was similar among healthy and BPH control groups. Since BPH is very common in the seventh to eighth decade of life, it would be almost impossible to identify men without BPH in this age group, and it is accepted that BPH is not a premalignant lesion.

Our results correlate with some and deviate from other population reports. The main factor that seems to explain the disparity in the results is the ethnogeographic origin of the various populations. The majority of published studies have analysed Caucasians but with different geographic origins and under different etiologies. Most studies reporting an association between short CAG repeats and high risk of PCa were on North Americans (Giovannucci et al. 1997; Ingles et al. 1997; Stanford et al. 1997; Xue et al. 2000; Modugno et al. 2001). However, our study, and studies on North Indian

(Mishra et al. 2005) and Chinese (Hsing et al. 2000) men suggest that even Asians, who are considered a low-risk group, have significant association between CAG repeat length variation and PCa risk. On the other hand, studies on European men, including British (Edwards et al. 1999), Swedish (Bratt et al. 1999), German (Correa-Cerro et al. 1999), and French (Latil et al. 2001), and on Australians (Beilin et al. 2001), found no association between CAG polymorphism and PCa risk. Gene-environment interactions may partially explain the different results obtained in each studied population. Differences in study design and reference CAG length may also contribute to the divergent results of epidemiological studies.

Shorter CAG repeat length has been associated with young age at diagnosis (Hardy et al. 1996; Bratt et al. 1999) and aggressive PCa phenotype in some studies (Giovannucci et al. 1997; Ingles et al. 1997). However, in our study we found no significant association between short repeats and age of onset or cancer grades as reported earlier (Lange et al. 2000; Hsing et al. 2000; Beilin et al. 2001; Mishra et al. 2005).

To the best of our knowledge, this is the first study to investigate the association between CAG repeat polymorphism and the relative risk of PCa in South Indian men, and our results suggest that, even in a low-risk population, shorter CAG repeats confer higher risk of PCa. However, further studies with larger series of cases, functional studies on AR polymorphism, and the role of CAG repeat length in response to endocrine therapy, may help determine the clinical significance of CAG repeats in the pathogenesis and prognosis of PCa. Further, early diagnosis would help in better management and appropriate treatment of PCa.

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