



Androgen receptor CAG polymorphism and the risk of benign prostatic hyperplasia in a Brazilian population

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ABSTRACT

Benign prostatic hyperplasia (BPH) is a very frequent age-related proliferative abnormality in men. Polymorphic CAG repeat in the androgen receptor (AR) can alter transactivation of androgen-responsive genes and potentially influence BPH risk. We investigated the association between CAG repeat length and risk of BPH in a case-control study of a Brazilian population. We evaluated 214 patients; 126 with BPH and 88 healthy controls. DNA was extracted from peripheral leucocytes and the AR gene was analyzed using fragment analysis. Hazard ratio (HR) and 95% confidence interval were estimated using logistic regression models. Mean CAG length was not different between patients with BPH and controls. The CAG repeat length was examined as a categorical variable (CAG \leq 21 vs. CAG $>$ 21 and CAG \leq 22 vs. CAG $>$ 22) and did not differ between the control vs. the BPH group. We found no evidence for an association between AR CAG repeat length in BPH risk in a population-based sample of Brazilians.

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INTRODUCTION

Benign prostatic hyperplasia (BPH) is a very frequent age-related proliferative abnormality in men (1). BPH is considered a progressive disease, defined as continuous growth of the prostate, leading to intensification of symptoms and increased risk of complications, such as increased risk of acute urinary retention and BPH-related surgery (2). Studies show that the prevalence of BPH is around 40 to 50% at the age of 50 years, and approximately 80% at the age of 70 (1,3).

The pathogenesis of tumor development has been closely associated with the action of steroid hormones (4,5). The androgenic effects are mediated by testosterone and dihydrotestos-

terone (DHT) in the target cells and their action has been demonstrated in the morphogenesis, differentiation, cell proliferation, and secretions of the prostate gland. Androgen binding promotes the activation of the androgen receptor (AR) and recruitment of co-factors, leading to the transcription of hormone-dependent target genes (6-9).

The human AR gene is located in chromosome X, on the q11-q12 region, which contains 8 exons (10) and has an approximate size of 90 kb (11). A critical function of the AR gene product is to activate the expression of other genes. The transactivation activity lies in the N-terminal domain of the protein (encoded by exon 1). Two polymorphic microsatellites are located approximately 1.1 kb away in exon 1: a highly

polymorphic CAG repeat and a less polymorphic GGC repeat (11,12). The CAG repeat encodes a polyglutamine tract; it ranges from 8 to 31 repeats and averages approximately 20 repeats (13).

In vitro studies have shown a negative correlation between the number of CAG repeats and the transcriptional activity of the AR. The increased number of these repeats reduces transcriptional activity in the AR, whereas a reduction to zero induces increased AR (11,14,15).

Therefore, the objective of the present study was to investigate whether CAG variant can be related to the development of BPH analyzing the frequency of AR CAG polymorphism in a sample of male individuals from southern Brazil.

MATERIALS AND METHODS

Study population

This case-control study was prospectively conducted at the Universidade Federal do Rio Grande do Sul from September 2004 to January 2009. The study was approved by the local and national Ethics Committee and informed consent was obtained from every subject. Prostate hyperplasia patients were selected from the Urology outpatient clinic at the Hospital de Clínicas de Porto Alegre. Inclusion criteria were age 40-80 years old, prostate volume larger than 30g (evaluated by abdominal ultrasound to define BPH group), no past or current hormone-ablation therapy or 5 α -reductase inhibitor therapy, and no concomitant neoplasia. Patients were submitted to surgery and the diagnosis of BPH was confirmed by pathological examination. Factors such as age at diagnosis, race (self-described), tumor stage and grade, total serum PSA (prostate-specific antigen) at diagnosis, and family history were recorded. Blood was collected to perform polymorphism analysis and to measure total serum testosterone. Controls were selected from a prostate cancer prevention program conducted since 2004 at the same institution. Inclusion criteria were age 40-80 years, prostate volume smaller than 30g, PSA value less than 2.0 ng/mL, normal digital rectal examination, and no concomitant neoplasia.

Genotyping

Genomic DNA for patients and controls was extracted from peripheral blood leukocytes. After erythrocyte lysis, leukocyte lysis was performed using 2 mL of specific solution (NaCl 150 mM, Tris-HCl 10 mM, pH 8.0; EDTA 10 mM, pH 8.0), 36 μ L 10% SDS, and 30 μ L of proteinase K (10 mg/mL), incubated at 37 °C for 18 hours. DNA was extracted and precipitated with 70% ethanol and re-suspended with specific buffer TE 10:0.1 (Tris-HCl 10 mM, pH 8.0; EDTA 0.1 mM, pH 8.0).

PCR was carried out at a final volume of 50 μ L. One μ L of the genomic DNA was denatured at 96 °C for 2 min in the presence of 20 mM Tris-HCl pH 8.4 plus 50 mM KCl and 1.5 mM MgCl₂. After this hot start, 1.25 U of Taq DNA polymerase was added together with the same Tris-HCl buffer, 1.5 mM MgCl₂, 0.4 μ M sense and antisense primers and 0.2 mM dNTP mix.

The primers used for polymorphism amplification were CAG primer 5'-TCCAGAATCT-GTTCCAGAGCGTGC-3' (forward) and 5'-GCT-GTGAAGGTTGCTGTTCCCTCAT-3' (reverse). Both sense primers were labeled with FAM fluorescent dye. Amplifications were performed using an automated thermal cycler (MJ Research, Waltham, MA, USA) applying the following conditions: hot start, 2 min at 96 °C; three cycles of 40 sec at 94 °C, 30 sec at 67 °C and 20 sec at 72 °C; three cycles under the same physical conditions except for the annealing temperature, which was 64 °C; three cycles at an annealing temperature of 61 °C; three cycles at an annealing temperature of 59 °C; and 25 cycles at an annealing temperature of 55 °C. The quality of the PCR products was assessed using 1.5% agarose gel electrophoresis. Each PCR product was diluted in water (10X) for analysis, and 2 μ L were mixed with deionized formamide and a fluorescent molecular weight marker [GeneMapper 500HD (ROX) Size Standard, Applied Biosystems, Foster City, CA, USA]. After denaturation for 1 min at 95 °C, each sample was submitted to capillary electrophoresis on an ABI 3100-Avant automated sequencer and the PCR products were analyzed with the GeneMapper software (Applied Biosystems, Foster City, CA, USA). The

number of CAG was calculated based on the size of the PCR products considering a series of standards obtained by direct sequencing of PCR products.

Statistical analysis

Differences between means in the continuous variables were analyzed by T test, with 95% significance. The genotype frequency between cases and controls was tested using standard χ^2 tests. Logistic regression was used to provide hazard ratio (HR), 95% confidence intervals (CI) and p-values for the risk of CAG repeat with BPH. The CAG repeat lengths were examined as categorical variables (CAG \leq 21 vs. CAG $>$ 21 and CAG \leq 22 vs. CAG $>$ 22). The categories were defined based on median analysis. Data analysis was performed using the computer software SPSS for windows (version 16.0).

RESULTS

The characteristics of the studied population are shown in Table-1. Genomic DNA from 126 BPH patients and 88 healthy male controls was ex-

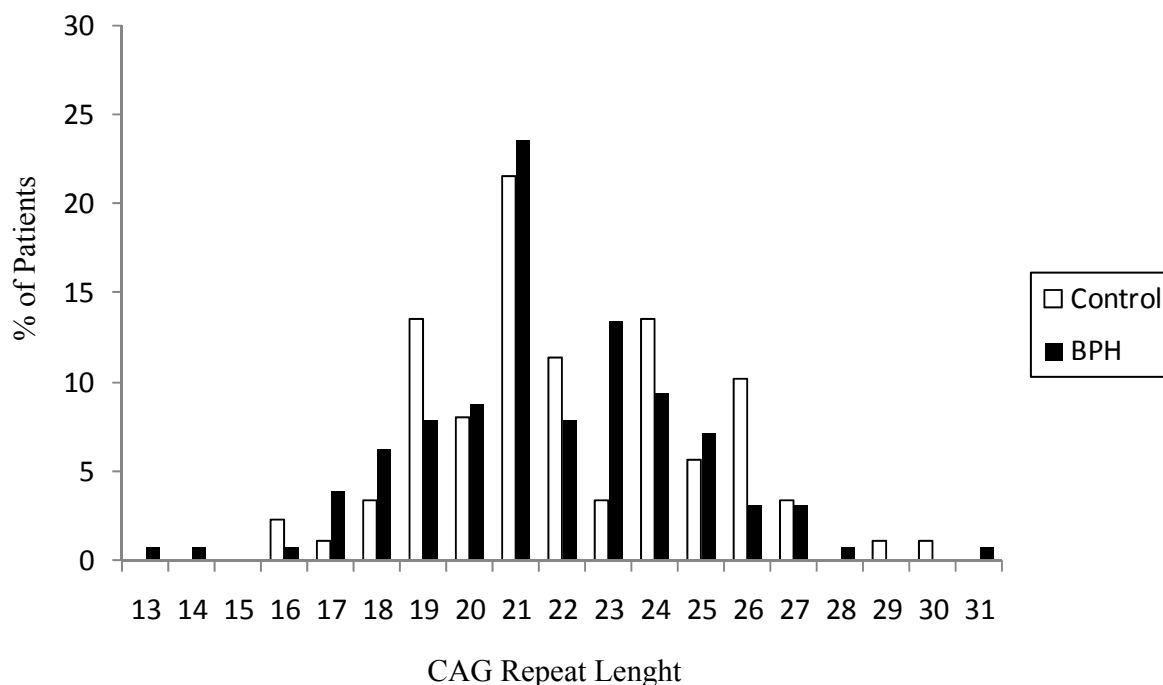
amined to determine the number of CAG repeats. The participant's mean age was 62.84 ± 9.15 in the BPH group and 56.68 ± 8.08 in the control group. BPH patients were older than controls ($p < 0.001$). There was a predominance of white individuals in both groups (89.7 BPH and 82.3% controls). Total serum PSA was 0.76 (0.55-1.05) ng/mL in the control group and 1.47 (0.69-3.94) ng/mL in the BPH group. PSA was not different between the groups ($p = 0.566$). The prostatic volume was 20.00 (14.38-23.94) cm³ in the control group and 35.68 (30.00-49.00) cm³ in the BPH group. Prostatic volume was higher in the BPH group than in the control group ($p < 0.001$). The mean number of CAG repeats in exon 1 of the AR was 22.11 ± 2.89 in the control group and 21.62 ± 2.84 in the BPH group (Table-1). No significant difference was found between the mean CAG repeats of cases and controls. The distribution frequency of the number of CAG repeat polymorphisms is shown in Figure-1.

Based on the median number of CAG repeats in the control group, the population studied was dichotomously classified into different subgroups, CAG \leq 21 vs. CAG $>$ 21 and CAG \leq 22 vs. CAG $>$

Table 1 - Characteristics of study population (n = 344).

	BPH (n=126)	Controls (n=88)
Age (years)^c		
Mean \pm SD	62.84 \pm 9.15	56.68 \pm 8.08
Range	41 - 82	41 - 75
Race (caucasians) ^a	113 (89.7%)	73 (82.3%)
PSA (ng/mL) ^{b,c}	1.47 (0.69 – 3.94)	0.76 (0.55 – 1.05)
Volume (cm ³) ^{b,c}	35.68 (30.00 – 49.00)	20.00 (14.38 – 23.94)
CAG repeat		
Mean \pm SD	21.62 \pm 2.84	22.11 \pm 2.89
Median	21	21.5
Range	13 - 31	16 - 30

Values are expressed as ^anumber of cases and (%), ^bmedian and percentile 25/75, ^cP<0.05.

Figure 1 - Frequency distribution of CAG repeats in the control and BPH groups.

22 (Table-2). According to this analysis, 66.3% of BPH group presented CAG > 21 and 56.9% presented CAG ≤ 21 ($p = 0.352$) and 58% of BPH group presented CAG > 22 and 58.8% presented CAG ≤ 22 ($p = 0.513$). The distribution frequency between the both groups was similar. There was no difference regarding any other dichotomic classification of the groups (data not show).

Considering that age is one of the major risk factors for prostate cancer and BPH development, we evaluated the risk of the genotypes de-

scribed above to develop these pathologies using logistic regression analysis, considering age as a continuous variable in the regression model (Table-3). The risk of developing BPH in individuals who have CAG > 21 compared to CAG ≤ 21 was 1.013 (95%CI 0.752-1.365; $p = 0.932$), whereas the comparison between individuals with CAG > 22 and CAG ≤ 22 showed a risk of 0.891 (95%CI 0.488-1.627; $p = 0.708$). Similarly, we found no difference regarding any other dichotomic classification of the groups (data not show).

Table 2 - Frequency distribution of CAG-21 and CAG-22 genotypes between BPH patients and controls.

	n	BPH n (%)	Controls n (%)	p*
CAG ≤ 21	102	58 (56.9%)	44 (43.1%)	0.352
CAG > 21	101	67 (66.3%)	44 (33.7%)	
CAG ≤ 22	131	77 (58.8%)	54 (41.2%)	0.513
CAG > 22	81	47 (58%)	34 (42%)	

Table 3 - Risk analysis for BPH of CAG-21 and CAG-22 genotypes

	BPH HR	95%CI	p*
CAG ≤ 21	1.013	0.752-1.365	0.932
CAG > 21			
CAG ≤ 22	0.891	0.488-1.627	0.708
CAG > 22			

DISCUSSION

Our results showed no association between AR CAG allele length and risk of developing BPH.

The AR is a transactivation factor that depends on the binding of a steroid hormone. This androgen-regulated transactivation activity is a key factor in the proliferation and differentiation of prostate cells. The polymorphic variation of the AR gene regulatory region, where the polymorphisms with the highest variation are located (CAG and GGC), may alter the transcriptional activity of the receptor (11).

In the present study, we found no significant difference in the number of CAG repeats between the control and BPH groups. The few studies that investigated AR CAG repeat and the risk to develop BPH have shown conflicting results. Our findings corroborate some data from the literature that also did not demonstrate differences between CAG repeat means in BPH patients and controls (16-18). AR CAG repeats were not associated with the risk to develop BPH, but shorter AR CAG repeats and PSA non-GG genotypes were significantly associated with decreased risk in BPH patients (19). However, in a recent case-control study of 416 BPH cases and 527 controls, CAG repeat length was associated with the risk of incidence of BPH (20). In another recent study conducted in Iranian patients, the mean number of CAG repeat in BPH patients was significantly smaller than normal (19.9 vs. 21.9; $p < 0.0001$) (21).

Studies suggest that the number of CAG and GGC repeats may be related to the ethnic group. In African-American populations, the mean number of repeats was demonstrated to

be lower than among Caucasians (13,22,23). The same was found in certain African subpopulations (13,23,24), while the Asian population would have a larger mean number of repeats (23,25). This might partly account for the frequency distribution of prostate cancer in the different regions of the world. In the present study, the proportion of black individuals was small and homogeneously distributed among the BPH and control groups. A separate analysis taking this factor into account did not show any differences. Moreover, in our study, information on race (or skin color) was collected by the examiner, a method that is not sufficiently adequate to define race/ethnicity. In fact, in Brazil as a whole, it is particularly difficult to assess race, due to the extensive genetic heterogeneity observed and the overlap of genetic characteristics among Europeans, Africans, and Native Americans (26). However, the patients' color distribution was the same as the one found by Parra et al., who analyzed skin color and genomic ancestry in Brazilians and found an European dominance in the south of Brazil (27). Germans and Italians are the main immigrant groups in Rio Grande do Sul, a southern Brazilian state (28). According to the last demographic census, 87.5% of the state population was classified as white, 5% as black, 7% as brown, 0.1% as Asian, and 0.4% as Amerindian; whereas in Brazil, 53.7% are white, 6.2% are black, 38.4% are brown, 0.4% are Asian, and 0.4% are Amerindians (IBGE Census 2000; <http://ibge.gov.br>).

It is believed that AR activity is inversely correlated with CAG repeat length based on investigations conducted using reporter-systems containing viral promoters (11,13,14,29). Furthermore, recent new results about AR activity and CAG repeats have been demonstrated. Nenonen et al. demonstrated that the CAG repeat number is not inversely associated with AR activity in vitro when analyzing CAG lengths within normal range (16,22 and 28) in a reporter-assay with the human PSA promoter as target. Using β -galactosidase as transfection control, 22CAG had the highest activity compared with 16CAG and 28CAG, whereas using renilla-luciferase the authors found that 16CAG behaved similarly to 22CAG and 28CAG, showing lower activity (30).

CONCLUSIONS

Our results suggest that specific haplotype of AR is not essential to develop BPH. In conclusion, our data suggest no evidence for an association between AR CAG repeat length and BPH risk in a population-based sample of Brazilians.

ABBREVIATIONS

AR - Androgen Receptor
 BPH - Benign Prostatic Hyperplasia
 CI - Confidence Intervals
 DHT - Dihydrotestosterone
 DNA - Deoxyribonucleic Acid
 HR - Hazard Ratio
 kb - Kilo-base Pair
 PCR - Polymerase Chain Reaction
 PSA - Prostate-Specific Antigen
 SPSS - Statistical Package for the Social Sciences

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CONFLICT OF INTEREST

None declared.

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