

The place of androgen receptor gene mutation analysis in the molecular diagnosis of prostate cancer and genotype-phenotype relationship

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Aim: To determine the relationship between androgen receptor (AR) gene polymorphism and prostate cancer in our society.

Materials and methods: Thirty-nine patients diagnosed with prostate cancer and 34 benign prostatic hyperplasia (BPH) patients who were diagnosed in 2010 met the study criteria. The inclusion criteria included patients whose diagnosis was confirmed with a biopsy, with the presence of adequate pathologic material for review, between the ages of 40 and 80, and who were healthy men without a family history of prostate cancer. The exclusion criteria excluded men diagnosed with another cancer and those who had kin with a history of prostate cancer. A direct DNA sequencing method was utilized for detection of polymorphisms.

Results: CAG repeat length varied from 13 to 28 (mean: 21.67) for the BPH group and 12 to 28 (mean: 21.74) for the prostate cancer group. Prostate-specific antigen (PSA) density and the androgen receptor (AR) CAG repeat had a statistically significant negative correlation in the BPH group. A statistically significant difference was associated between AR CAG repeat and PSA density.

Conclusion: Randomized prospective studies should be planned with larger patient and control groups and with more variables, which may open new horizons in prostate cancer screening and early detection.

Key words: Androgen receptor, mutation, polymorphism, prostatic hyperplasia, prostatic neoplasm

1. Introduction

Prostate cancer is the most common type of cancer in men in all countries of the world and ranks third among the causes of death resulting from cancer, after lung and colon cancers. This difference is due to known genetic and environmental factors (1,2).

Androgen receptors (ARs), by initiating the transcription of genes responsible for androgen, will add to the activity of testosterone and dihydrotestosterone. The AR gene includes 2 polymorphisms involved in the initiation and progression of prostate cancer. Exon 1 contains the 2 polymorphic trinucleotide repeat encoding polyglutamine (CAG)_n (3). Polymorphic repeat length is inversely related to the AR gene transcription activity (e.g., it regulates androgen receptors' response to androgens) (4).

Short CAG repeat lengths have been proposed as increasing the androgen activity organized by AR, leading to increased sensitivity to prostate cancer and benign prostatic hyperplasia. In line with this hypothesis, numerous epidemiological studies found an association between the CAG repeat length and prostate cancer risk

and cancer aggressiveness (5). In men with CAG repeat lengths of less than 18, as compared to those with repeats of longer than 26, the relative risk (RR) of prostate cancer was found to be more than 1.5-fold. Shorter CAG lengths are associated with advanced stage (RR: 2.2) and high-grade disease (RR: 1.9) (6,7).

In studies of men without prostate cancer, short CAG repeat length prevalence was found to be highest in African-Americans, moderate in non-Hispanic whites, and lowest in Asians (6). These data suggest that ethnic differences in the incidence of prostate cancer may be partly explained by AR polymorphism.

In this prospective study, we aimed to evaluate the relationship between AR gene polymorphism and prostate cancer in our society. The detection of AR gene variants may play an important role in the early diagnosis and treatment of prostate cancer, and could help relate it to the clinical phenotype and provide the genotype-phenotype correlation. Our main goal was to make use of them as prognostic indicators.

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2. Materials and methods

Thirty-nine patients diagnosed with prostate cancer (PCa) and 34 patients diagnosed with benign prostate hyperplasia (BPH) were included in this single-center, multidisciplinary, prospective study. Volunteers in both groups were between 40 and 80 years old and the diagnoses (PCa and BPH) were confirmed by biopsy.

Prostate-specific antigen (PSA) was measured as ng/mL. The volume of the prostate was calculated by transrectal ultrasound (TRUS), and for the fraction of patients who had not received TRUS, it was calculated as an average by digital rectal examination.

Direct DNA sequencing is a commonly used method for the detection of CAG repeat expansion in the first exon of the AR gene (5). We also benefited from this method. After obtaining approval from the ethics committee, all patients who participated in this study filled in a consent form. A 1-mL blood sample was taken from all individuals who agreed to participate in the study and placed into tubes containing ethylenediaminetetraacetic acid to obtain genomic DNA for analysis of AR gene CAG repeat mutations.

2.1. Genomic DNA extraction

Extraction of total genomic DNA from peripheral blood leukocytes was performed with the unsalted column purification method. A QIAGEN Mini Blood DNA Isolation Kit was used for this purpose. For measuring the purity of DNA, the DNA solution absorbance intensity was measured with a NanoDrop Spectrophotometer device at a wavelength of 280 nm. DNA quality level was determined by electrophoresis of 2 μ L (100 ng) of DNA in 1% agarose gel.

Genotyping analysis of the AR gene polymorphisms were performed by polymerase chain reaction (PCR) followed by a DNA sequencing analysis method. First, the AR gene PCR amplification reactions were performed.

For this purpose, a mixture of 100 ng of genomic DNA, 1X PCR buffer (10 mM Tris-HCl, 50 mM KCl, pH 8.3), 1.5 mM MgCl₂ (Applied Biosystems), 200 mM each of deoxynucleotide triphosphates (dNTPs) (Promega, Madison, WI, USA), 1 μ M sense and 1 μ M antisense primers (Invitrogen Ltd., Paisley, UK), and 1 U AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA, USA) was completed to 25 μ L with deionized water and subjected first to a total of 10 min denaturation at 94 °C followed by 35 cycles at an annealing temperature of 60 °C, and PCR was completed at 72 °C for 10 min and then at 4 °C (endless process). The obtained PCR amplification products were checked by agarose gel electrophoresis. Before cycle sequencing reactions, the amplified PCR products were purified using an Exo-SAP PCR purification

kit (Amersham Life Science). Cycle sequencing PCR was performed using a BigDye Terminator v.3.1 kit as per manufacturer's instructions (Applied Biosystems). Cycle sequencing PCR products, after purification with a BigDyeXT Terminator kit (Applied Biosystems), were analyzed using an ABI 3130xl genetic analyzer system. DNA sequencing was performed in both directions, initiated from the forward and the reverse primers used in the initial PCR reaction (Figures 1 and 2). For sequence evaluation, the SeqScape 2.0 sequencing analysis software was used.

2.2. Statistical analysis

All data were prospectively collected. Descriptive statistics, Student's t-test, and Pearson's correlation analyses were performed. Significance was defined as $P < 0.05$.

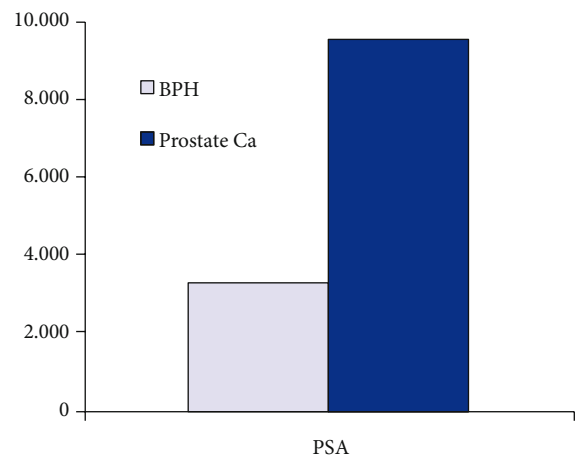


Figure 1. DNA sequence electropherogram for CAG repeats of (28 repeats) AR gene exon 1.

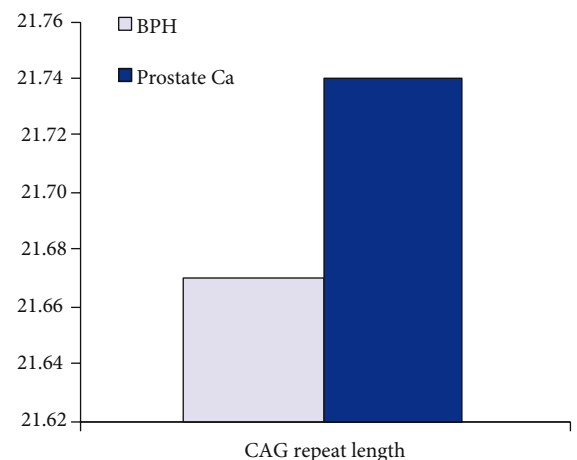


Figure 2. DNA sequence electropherogram for CAG repeats of (11 repeats) AR gene exon 1.

3. Results

A total of 73 patients, with 39 prostate cancer and 34 BPH patients, were enrolled in the study. All patients were diagnosed in 2010. Age, PSA, prostate volume, PSA density (PSA value to prostate volume ratio), and the number of AR CAG repeats were compared between the 2 groups. The Gleason score and the family history of prostate cancer were also evaluated in the prostate cancer group. While the average age of diagnosis for the BPH group was 69.45 years, it was 63.90 for the cancer group. While PSA varied between 0.2 ng/mL and 27.7 ng/mL (mean: 3.282 ng/mL) for the BPH group, it varied between 0.015 ng/mL and 101.9 ng/mL (mean: 9.510 ng/mL) for the cancer group (Figure 3).

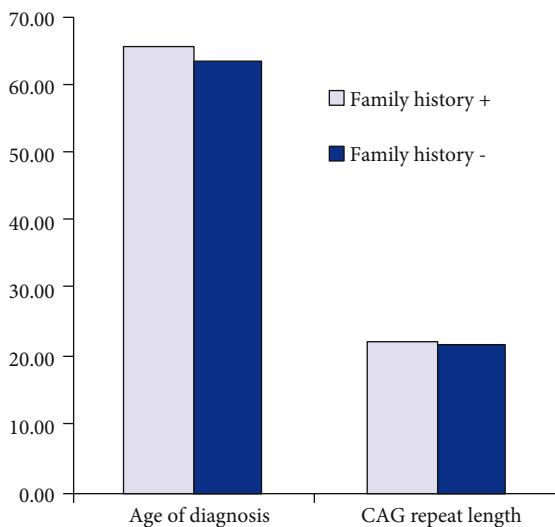


Figure 3. PSA comparison between groups.

While prostate volume varied from 21 mL to 52 mL (mean: 32.42 mL) for the BPH group, it varied between 21 mL and 75 mL (mean: 41.21 mL) for the cancer group. While PSA density varied from 0.005 to 0.630 (mean: 0.093) for the BPH group, it varied between 0.001 and 1.644 (mean: 0.208) for the cancer group.

CAG repeat length varied from 13 to 28 (mean: 21.67) for the BPH group and varied between 12 and 28 (mean: 21.74) for the prostate cancer group (Figure 4).

PSA density and the androgen receptor CAG repeat length had a statistically significant negative correlation in the BPH group (Spearman's rho = -0.484, $P = 0.004$).

The androgen receptor CAG repeat length in the cancer group was negatively correlated with the Gleason score, but was not statistically significant (Spearman's rho = -0.049, $P = 0.765$). A statistically significant difference was associated between androgen receptor CAG repeat length and PSA density (Spearman's rho = 0.321, $P = 0.046$).

While in the group with family history of prostate cancer the average age at diagnosis was 65.46 years, it was 63.12 for the patients without a family history. PSA density was 0.170 in the group with a family history, and it was 1.227 in the group without a family history. CAG repeat length was 22.15 in the group with a family history, while it was 21.54 for the patients without a family history (Figure 5).

A positive correlation was determined between PSA and AR CAG repeat length, although it was not statistically significant (Spearman's rho = 0.207, $P = 0.206$).

4. Discussion

Prostate cancer is the fourth most common male malignancy in the world. Incidence and mortality rates vary greatly among countries. This variability is very similar to changes, as observed in the United States,

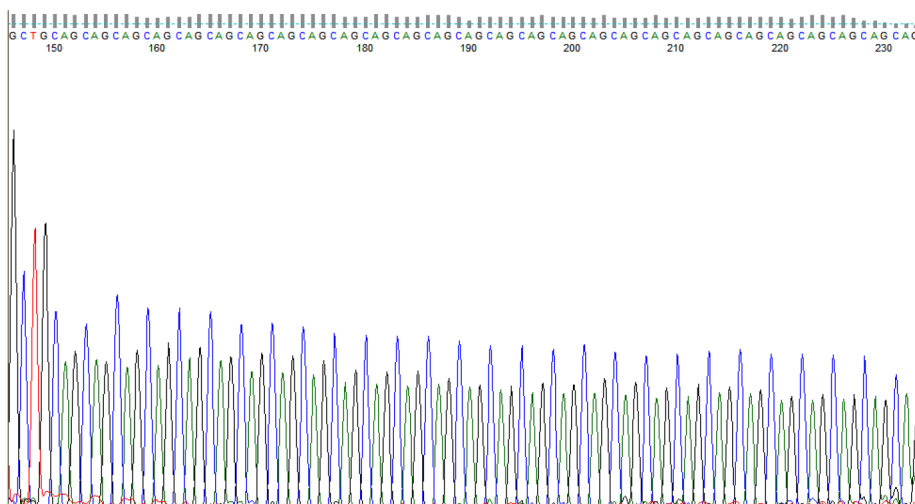


Figure 4. CAG repeat length comparison between the groups.

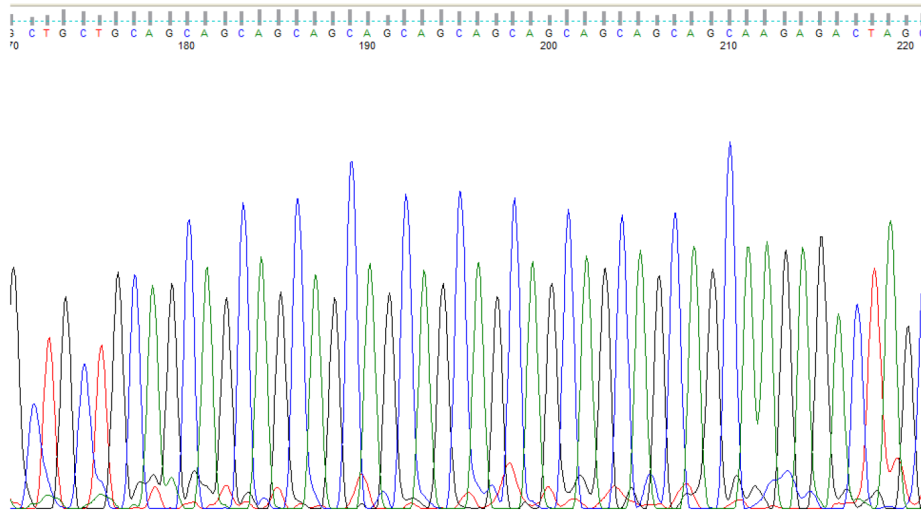


Figure 5. Comparison between family history with age of diagnosis and CAG repeat length.

between different ethnic groups. In the United States prostate cancer is the second most common cause of cancer diagnosis and cancer-related deaths among males. Incidence and mortality rates are generally higher in Western countries than developing countries. Since the beginning of 1990, new screening tests and treatment improvements have led to a dramatic amelioration in the incidence, mortality, and diagnostic phase of this disease. Huge advances in molecular biology and epidemiology have opened new horizons in the etiology and biology of prostate cancer. These developments will lead to a better understanding of this disease and will open new and better ways for the prevention and treatment of prostate cancer in the foreseeable future.

Many authors are in consensus about the fact that aggressive screening and treatment is responsible for the decrease in prostate cancer mortality. This result seems especially plausible considering that prostate cancer mortality rates have not decreased in the countries where the PSA test has not yet begun to be used as routine. The role of PSA has been clearer as a result of prostate, lung, colon, rectum, and ovarian cancer screening trials as the scan reached a certain maturity.

The etiopathogenesis of prostate cancer is largely unknown, but the available data suggest that the disease is multifactorial (8). Androgens are the main male sex hormones and manage male secondary differentiation, spermatogenesis, and development of the accessory sex organs, including the prostate gland. Androgens act through the AR protein, which is a ligand-dependent nuclear transcription factor. The role of androgens in prostate cancer has not been fully elucidated yet. If the AR has oncogenic potential, androgen may play a role in initiating prostate cancer. The AR gene is mapped on the long arm of

the X chromosome (Xq11.2-q12). The N-terminal region of AR gene first exon is highly polymorphic, containing a variable number of CAG and GGC repetitions and codes for polyglutamine and polyglycine tracts (9). Age is the most important risk factor for PCa and BPH in all populations studied. There are studies indicating that a short CAG repeat length constitutes an increased risk for PCa and BPH (10), while a similar number of studies state that there is no relation between the repeat length and PCa risk (11). However, increasing CAG repeats have been associated with androgen insensitivity, testicular atrophy, and infertility (12). Experimental studies found an inverse relationship between the transactivation activity of the receptor and CAG repeat numbers (8). A typical distribution of the CAG repeats varies between 11 and 31 (13). It is shorter in African-Americans (20 repeats) than whites (22 repeats) (13). Many, but not all, epidemiological studies emphasized that shorter androgen receptor gene CAG repeat numbers were related to a higher risk of prostate cancer (13,14).

Prostate cancer risk may be inversely related to the number of CAG repeats. Coetzee and Ross suggested that the variations of CAG repeat length were associated with prostate cancer risk (15). Accordingly, most descriptions are concerned with short alleles with polyglutamine tract glutamine residues on the receptor, which can do more high transactivation, depending on the inverse relationship with transcriptional activity (15). This potential relationship can be modified with the stage/degree at diagnosis (16) and the men who were diagnosed at more advanced ages seem to have longer CAG repeats (17), while 2 studies found no statistically significant relationship between short CAG length and formation of prostate cancer (18,19). Bratt et al. (11) determined the relationship between CAG repeats

and diagnosis at a young age, but did not find a relationship with high risk. Eeles et al. (12) found no correlation between CAG repeat number and cancer development and aggressiveness in their study consisting of 178 cases of Caucasian patients and controls. To date, many studies have reported a positive association between short CAG repeat numbers and prostate cancer. On the other hand, the potential importance of the relationship is relatively limited, because of the limited sensitivity of the published results due to their small case groups.

With regard to the severity of prostate cancer, Giovannucci et al. (5) identified an inverse relationship between CAG repeat number and grade/degree. Stanford et al. (7) found that shorter repeats involve a higher risk for disease severity compared with longer ones. These results demonstrated that short repeats are not only associated with the development of cancer but are also associated with the potential severity of the cancer.

Decreased repeat length may make the prostate more vulnerable to chronic androgen stimulation and may result in an increase in consequent proliferative activity and somatic mutation rate (20). AR repeat polymorphisms have been associated in the incidence of other androgen-dependent clinical conditions, such as a high number of repetitions adversely affecting fertility, spermatogenesis, and bone density. A low number of repeats is associated with an increased risk of baldness and, occasionally, BPH (21).

Fewer studies have reported about the AR CAG repeat and BPH connection than the connection with cancer. No association between CAG repeat polymorphism and the risk of BPH was found in a population in the Netherlands (22).

Prostate cancer incidence and mortality differences among blacks, whites, and Asians may be explained by nutritional and socioeconomic factors, but to fully explain this difference, genetic components are required. Short CAG repeats are associated with high histologic grade, extracapsular extension, and a metastatic phenotype explained as an aggressive prostate cancer; however, this connection is controversial. Giovannucci et al. (5) found that the patients with the shortest length of CAG repeats have the most risk of cancer, while some studies could not determine any connection between the number of CAG repeats and tumor grade, stage at diagnosis, and PSA value (20).

For our study, CAG repeat length for the BPH group ranged between 13 and 28 (mean: 21.67), while it ranged between 12 and 28 (mean: 21.74) for the prostate cancer group. The number of repetitions for the prostate cancer

group was a little higher, although the difference was not statistically significant.

We detected a statistically significant negative correlation between PSA density and androgen receptor CAG repeats in the BPH group. The literature includes a limited number of studies on the relationship between BPH and the number of CAG repeats. While no association was found between the CAG repeat polymorphism and the risk of BPH among the population of the Netherlands, in the Finnish population short CAG repeats were rare in patients with BPH, similar to our study (17,22).

The androgen receptor CAG repeat length was negatively correlated with the Gleason score in the cancer group, but that was not statistically significant. This result lends support to the theory that a shorter CAG repeat sequence is associated with extraprostatic extension, distant metastasis, or high degree, as mentioned previously by Lai et al. (20). The relationship between androgen receptor CAG repeats and PSA density was found to be statistically significant. A positive correlation was found between PSA and androgen receptor CAG repeat length, although it was not statistically significant. Two studies in the literature found no connection between the PSA value and the number of CAG repeats (23,24). While PSA density was 0.170 in patients with a positive family history, it was 0.227 in the group without history. The CAG repeat length was 22.15 in the group with a positive history and was 21.54 in the group without history. Positive correlation was obtained between PSA and androgen receptor CAG repeat length, although it was not statistically significant.

Our study, examining the link between the number of CAG repeats and prostate cancer risk and other factors, is the first for Turkish society. By increasing the number of patients, and with the addition of other parameters, especially serum testosterone levels, different correlations can be analyzed and the meaning and importance of CAG repeat number in prostate cancer for the Turkish population can be assessed in more detail. As outlined, an evidence-based CAG repeat length test in the general population has not been verified yet. These results have shown that there is a need for additional studies on this subject. The relationship between AR CAG repeats and prostate cancer is interesting, but is limited to some extent. For a better understanding of prostate carcinogenesis we have to fully explain the relationship between genes associated with CAG repeats and environmental factors that increase the risk of prostate cancer. As a result, our ability to prevent prostate cancer will increase and it will provide more useful information in new diagnoses.

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