Polymorphism AR-E211 G>A of the AR Gene (G1733A) In Patients with Prostate Cancer (Pca) In Middle Euphrates of Iraq

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Abstract

The pathogenesis of prostate cancer (CaP) involves alterations in a gene structure of the androgen receptor (AR). The single nucleotide polymorphism AR-E211 G>A localized in exon 1 of the AR gene (G1733A) was detected using direct polymerase chain reaction and restriction digestion (PCR-RFLP) method on blood samples with prior DNA isolation. We used blood samples of patients with a diagnosis of CaP. From monitored group of CaP patients were selected specimen in formalin-fixed paraffin-embedded tissue blocks with morphology of CaP. The main objective of our study was to develop a method based the direct PCR-RFLP analysis from blood with prior DNA isolation for faster genotyping analysis of a large number of samples. We found no statistically significant differences in allelic % of the AR-E211 G>A polymorphism between healty and CaP patients. Genotyping of the AR-E211 G>A variant in blood was not identical with tumor tissue genotyping analysis. Although we analyzed a limited number of the patient samples, we suppose that a presence of the minor allele A may be associated with cancer transformation-induced changes of the modified AR gene.

1. Introduction

The prostate is both an accessory gland of the male reproductive system and a muscle-driven mechanical switch between urination and ejaculation. The prostate gland is of the male reproductive system. In adults, it is about the size of a walnut [1]. The prostate gland starts to develop laterally as epithelial buds from the urogenital sinus wall. These buds branch into solid cords which canalize to form the ducts and acini. The surrounding urogenital sinus mesenchyme forms the inter fascicular fibroblasts and the smooth muscle of the prostate Review of Prostate Anatomy and Embryology[2]. With androgenic stimulation of the androgen receptor expressed in prostatic Mullerian mesenchyma, the prostate start to forms [3]. The prostate is completely dependent on testicular androgens for both its development and the maintenance of its structural and functional integrity [4].

Prostate cancer is the second most common non-cutaneous cancer in men and a leading cause of death, with an estimated 174,650 new cases and 31,620 deaths in the [5]. Age and family history are key risk factors for prostate cancer, and black men have a higher risk of prostate cancer incidence and death compared to men from white or Asian backgrounds [6].

Prostate cancer is in fact very heterogeneous and potentially multifocal. Age and ethnicity are among the most important risk factors [7, 8]. In addition to these factors, a well-established risk factor is genetic predisposition. Throughout most of the years, several essential inherited elements in PCa susceptibility have been identified in genetic-epidemiological studies [9].

AR activity is intimately linked to prostate cancer, of the 1029 mutations found in gene that encodes the AR, 159 mutations predispose males to prostate cancer.

Prostate cancer cells, like normal prostate cells, require androgens to grow and survive. Growth of prostate cancer depends on the ratio of the rate of cell proliferation to the rate of cell death [10]. Androgen receptor (AR) has a role in the normal growth and development of the prostate gland, in prostate carcinogenesis and androgen-dependent (AD) or androgen-independent (AI) progression of the disease. Functional AR is expressed during various stages of prostate carcinogenesis from the very early stage of prostate intraepithelial neoplasia to organ-confined or locally invasive primary tumors, in metastatic tumor and before or after androgendeprivation therapy (ADT) [11-15]. In the adult prostate, the AR continues to be critical for maintenance of the organ. Unlike in the developing prostate, AR in the adult organ is primarily expressed in the prostatic luminal epithelial cell rather than stromal cell. At any age, depletion of testosterone through surgical or chemical castration causes the prostate to involute and decrease in size due to loss of secretory luminal epithelial cell. The prostate has incredible self-renewal potential, since many cycles of involution followed by restoration with androgen supplementation demonstrated that the organ can continuously regenerate for its original size [16, 17].

The frequency distribution of the AR gene CAG repeats also varies among different racial/ethnic groups and the ethnicity seems to be a significant risk factor for prostate cancer. Shorter alleles are found more frequently in African-American men who have a higher incidence of prostate cancer. On the

doi.org/10.31838/hiv22.02.352 Received: 05.04.22, Revised: 05.05.22, Accepted: 16.08.22

other hand, Caucasian and Asian populations at lower risk of prostate cancer, exhibit a relatively high number of CAG repeats [2, 3]. The G1733A single nucleotide polymorphism of the AR-E211 gene (UCSC code: rs6152), designated the G and An alleles is a synonymous change. The presence of the G allele at nucleotide 1733 abolished a StuI restriction enzyme recognition site, which is recognized on the A allele.

2. Methods

Blood samples were obtained from 90 patients at the 54–88 ages diagnosed with histopathological confirmed prostate cancer (CaP). The CaP patients were recruited from Department of Urology and archived in Department of Clinical and Molecular Pathology of the Imam Hussein (peace be upon him) Teaching Hospital in the Holy Karbala Governorate and Merjan Medical City in Babylon Governorate/ Iraq.

Ninety candidates of this group have neither symptoms nor signs of essential PCa by doing PSA serum level with per rectal examination of prostate and they are healthy otherwise. Data was collected by ways of a personal interview for every individual to obtain information about their smoking status and about their age, ethnicity and family history.

Almost 10 ml venous blood was drawn from each candidate when visit the oncology center for treatment. Two ml of this sample were collected in EDTA tube for DNA extraction and PCR. The remaining was transferred into a clean plain tube, and left at room temperature for nearly thirty minutes for clotting, then centrifuged. Serum was divided into two parts one for AR fig.1

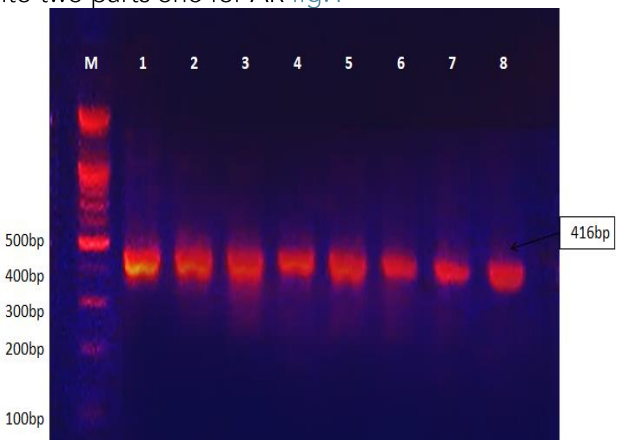

Figure 1 showing Electroph. For pcr prod. To the AR gene on 70 volt for 1 hr 3% agaros.

The PCR-RFLP (Restriction Fragment Length Polymorphism) assay was performed from blood samples without DNA purification. In the first step, genomic DNA was amplified directly from 2 µl whole blood using Finnzymes's Phusion® Blood Direct PCR Kit (Finnzymes, Espoo, Finland). The AR-E211 G>A polymorphism was detected by amplification of a 416 bp fragment using for-ward primer 416/F 5′- CAC AGG CTA CCT GGT CCT GG –3′ and reverse primer 416/R 5′- CTG CCT TAC ACA ACT CCT TGG C – 3′ [18, 19] on high-speed Piko® Thermal Cycler (Finnzymes, Espoo, Finland) by initial denaturation at 98 °C for 5 min, followed by 30 cycles of denaturation at 98 °C for 5 s, annealing at 58 °C for 4 s, elongation at 72 °C for 30 s, and the final extension at 72 °C for an additional 3 min after the last cycle. After PCR, the reactions were centrifuged at 2,500 rpm for 3 min and the supernatants were collected for restriction digestion. The digestions were prepared from 5 µl of supernatants by addition of 10 units of StuI (New England Biolabs, Ipswich, MA) and incubated at 37 °C for 1 h. Digested products were electrophoresed through a 3 % agarose gel and visualized by ethidium bromide staining fig. 2

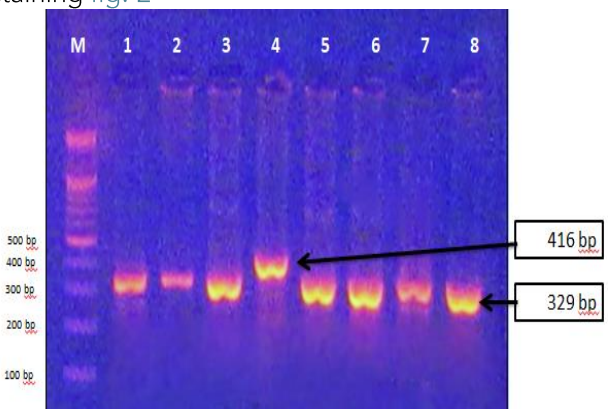

Figure 2 PCR-RFLP after added cutting enzyme.

3. Result

It is still studied by the assumption that the minor allele A is associated with higher risk of prostate cancer [20]. Our finding indicates that the minor allele A could be associated with transformation-induced changes of the modified androgen receptor gene or induced another changes caused by tumor transformation. Frequency of Alleles of the AR-E211 G>A Polymorphism in Blood Samples. Since the AR gene is located on the X chromosome, males have only one allele on their one X chromosome and females have two alleles on their two X chromosomes [21]. The PCR-RFLP assay was performed from blood samples without DNA purification. In the first step, genomic DNA was amplified directly from 2 µl whole blood, the AR-E211 G>A polymorphism was detected by amplification of a 416 bp fragment using forward primer 416/F 5′- CAC AGG CTA CCT GGT CCT GG –3′ and reverse primer 416/R 5′- CTG CCT TAC ACA ACT CCT TGG C – 3′ (Fig. 1).

Comparison of allele frequencies between the groups of CaP patients and between the groups of control were analyzed by $\chi 2$ – tests. The genotypes association was shown the following results and can also show in Table (1). The model codominant was non-significant change with (P = 0.7) when compared with healthy control. The model dominant was shown non-significant change with (P = 0.7) when compared with healthy control according to the percentage of A/G –G/G is genotypes response and related with disease RA. The recessive model was shown non-significant change with (P = 0.7) when compared with healthy control.

Table 1 Allelic Association of AR Gene Polymorphism in Prostate Cancer Males Cases Versus Controls Males Patients.					
Model	Genotype	Case	Control	OR (95% CI)	P-value
Codominant	A/A	8 (8.8%)	7 (7.7%)	1.15(0.401-3.33	0.7
	A/G	0 (0%)	0 (0%)		
	G/G	82 (91.1%)	83 (92.2%)		
Dominant	A/A	8 (8.8%)	7(7.7%)		0.7
	A/G-G/G	82 (91.1%)	83 (92.2%)	1.15(0.401-3.33)	
Recessive	A/A-A/G	8 (8.8%)	7(7.7%)		0.7
	G/G	82 (91.1%)	83 (92.2%)	1.15(0.401-3.33)	
Overdominant	A/A-G/G	90 (100%)	90 (100%)		
	A/G	0 (0%)	0 (0%)		

The 416 bp fragment digested by cutting enzyme to two bands 329 bp band and 87 bp band. The electrophoresis shows the picture with 416 band not cut by enzyme indicate A/A polymorphism homozygous-undigested and 329 bp band indicate G/G polymorphism while the 87 bp band was small not appear, so any patient sample has 416 bp and 329 bp indicate heterozygous, patient contain 416 bp only dominant, while who contain 329 bp only as 87 bp not appear was homozygous- digested.

4. Discussion and Conclusion

Several studies on mutations in the AR gene in human prostate cancer have already been reported. Yang et al. [22] found that 25% of advanced lesions had a mutation at codon 877, which lies within the hormone-binding domain. This was the same as that detected in LNCaP. However, the CAG and GGC polymorphic repeats e nucleotide in the AR gene have been studied extensively as markers of prostate cancer susceptibility, with inconclusive results [23-25].

Given the AR gene is highly conserved, with only a single dimorphic marker reported in Caucasian populations, we investigated the role of this polymorphism on androgen related prostate cancer and risk factors for prostate cancer (age, country of birth, family history. As for the number of CAG repeats in the AR gene. Rutkowski [26] reported a contraction of CAG repeats in a prostate cancer case which showed a paradoxical agonistic response to hormone therapy with an anti-androgen flutamide.

The E211 G>A is in partial linkage disequilibrium with both CAG and GGC repeats [24], it's used as the genetic marker has been limited. It is well established that nucleotide repeat sequences are highly polymorphic that can be reflecting a high rate of mutation [27], whereas dimorphic polym orphisms are more stable that can be reflecting lower rates of mutation, and thus ideal markers in association studies. We therefore utilized the E211 marker to ascertain association with prostate cancer and known risk factors.

Result of the genetic analysis of the AR-E211 G>A polymorphisms of patients that confirmed with histological study has prostate cancer was association of the A genotypic variant we found no overall association between the presence of the A allele and CaP patients with Gleason grades. So many far studies have been carried out searching for a relationship between the AR-E211 G>A polymorphism detected in blood and various degree changes in tumor prostatic tissue [32].

Although, we analyzed only a small sample of patients, in several cases we have repeatedly identified in non-tumor and tumor tissues, both alleles of the AR-E211 G>A polymorphism. It is still studied by the default that the minor allele A is associated with higher risk of prostate cancer [28]. Our finding indicates that the minor allele A could be associated with transformation-induced changes of the modified androgen receptor gene or induced another changes caused by tumor transformation.

In our study, the A allele is not associated with overall prostate cancer risk, but decreases the risk of metastatic disease. Shorter CAG repeats have been reported to be more common in prostate tumors and older age at diagnosis.

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