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IGF1 CA-Repeat Polymorphism and Prostate Cancer Development in Isfahan Province of Iran

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Abstract

Background: Prostate cancer is increasing among Iranian men and gene polymorphisms may play a role in the development of prostate cancer. Insulin-like growth factor 1 (IGF1) gene polymorphisms have been deeply explored in different malignancies. In this study, we aimed to explore the association of IGF1 CA repeat length polymorphism with the risk of prostate cancer development in Isfahan province of Iran.

Method: The total blood of 100 prostate cancer patients and the equivalent matched control individuals were collected. DNA extraction was followed by IGF1 promoter polymorphism amplification. Genotyping was performed using polyacrylamide gel electrophoresis and sequencing was performed.

Results: According to the results, IGF1 promoter polymorphic site showed six different alleles ranging from 17-22 CA repeats among our studied population. Comparing SL heterozygotes with both homozygotes, a significant increase in RR value (RR=4.5, p=0.031) was observed. Although age adjustment and family history did not elevate the RR value, but a significantly elevated risk of prostate cancer (RR= 3.143, p=0.002) was shown when we compared SS patients with LL ones according to their BPH history.

Conclusion: In conclusion, carriers of $(CA)_{17}$ allele could be at a higher risk of prostate cancer development and being SL heterozygotes could increase the risk of BPH development in our studied population.

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Introduction

Prostate cancer is at the top point of attention as the second most prevalent cancer and the third cause of death in our high-risk geopolitical region, the Middle East (1). Prostate cancer as a slow-growing malignancy and with an annual percentage rate of 17.3% has started to increase its risk of incidence among Iranian population (2, 3). Genetic factors such as vitamin D pathway genes and genes of cell cycle control angiogenesis and cell adhesion. Also, chromosomal loci have been shown to play a role in prostate cancer development and metastasis (4).

On the other hand, gene polymorphisms are thought to be able to predispose the incidence of various cancers (5). However, different racial and geographical studies show conflicting results concerning the impact of gene polymorphisms on prostate cancer development (6). Insulin-like growth factor 1 (IGF1) polymorphisms have been deeply explored in breast cancer (7, 8). Our previous study also declared a novel association between IGF1 CA repeat length and the risk of breast cancer development in Isfahanian women (9). In the present study, we tried to discover any possible association between IGF1 CA repeat length polymorphism and the risk of prostate cancer development in Isfahanian men. In addition, we tried to find any possible relationship between the age of onset or benign prostatic hyperplasia (BPH) and the risk of prostate cancer development.

Methods

A total of 272 participants including 136 men with prostate cancer and the equal number of matched healthy individuals without any kind of cancer in their family history, from Seyedoshohada Cancer Hospital, Isfahan University of Medical Sciences, Isfahan, Iran, entered the study. The purpose of the study was explained to all participants and informed consent was granted before blood donation. Data were collected by expert counselors in Seyedoshohada Cancer Hospital.

DNA extraction and Genotyping

DNA extraction and IGF1 promoter polymorphism amplification using predefined primers were performed as described previously (9). All materials were obtained from Sinaclone Company (Tehran, Iran). In optimized PCR conditions, including 2mM of $MgCl_2$, pre-denaturation was performed at 95°C for 5 min and it was followed by 33 cycles at 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min. A final cycle of extension at 72°C for 10 min was used for the completion of nascent products. PCR products were visualized on non-denaturing polyacrylamide gel electrophoresis (non-denaturing PAGE) coupled with silver staining as described previously (9). Alleles of variable sizes were sequenced directly form polyacrylamide gels and specific allelic markers were constructed for exact determination of CA repeat length of all samples.

Statistical analysis

All analyses were performed using SPSS software (IBM SPSS Statistics V21.0). To check the Hardy–Weinberg disequilibrium, Fisher's exact test was performed and *p*-values less than 0.05 were considered as significant. The presence or absence of correlations and the risk of prostate cancer development were assessed using risk ratio (RR) with 95% confidence intervals (95% CI).

Results

IGF1 genotyping and risk assessment

Allelic distribution of IGF1 promoter polymorphic site cleared six different alleles, ranging from 17-22 CA repeats, among our studied population (Table 1). Accordingly, the smallest allele with seventeen and the largest with twenty-two repeats of CA-dinucleotide were absent in control individuals (Table 1). Additionally, the most frequent allele among both patients and control individuals was $(CA)_{19}$ with 78.68% and 81.99% frequencies respectively. The most frequent allele was followed by $(CA)_{18}$ allele in both groups with a same frequency of 8.82% (Table 1). Alleles with 18, 19 and 20 CA repeats showed RR close to 1, but super-large $(CA)_{21}$ and $(CA)_{22}$ alleles tended to show an elevated insignificant RR values of 1.341 and 2.007 respectively (Table 1). As indicated in table 1, IGF1 $(CA)_{17}$ as our super-small allele, has increased the RR value more than two folds significantly (RR>2.019, p=0.030).

Table 1. Allelic distribution of IGF1 promoter polymorphic site in Iranian men

allele	No of patients (%)	No of control individuals (%)	Risk ratio (95% CI)	p value
$(CA)_{17}$	5 (1.84)	0 (0.00)	2.019 (1.854- 2.198)	0.030
$(CA)_{18}$	24 (8.82)	24 (8.82)	1.000 (0.744-1.345)	0.560
$(CA)_{19}$	214 (78.68)	223 (81.99)	0.903 (0.741-1.043)	0.332
$(CA)_{20}$	21 (7.72)	22 (8.09)	0.975 (0.709-1.340)	0.625
$(CA)_{21}$	6 (2.21)	3 (1.10)	1.341 (0.838-2.145)	0.252
$(CA)_{22}$	2 (0.74)	0 (0.00)	2.007 (1.845-2.184)	0.250
total	272 (100)	272 (100)		

Genotype distribution of IGF1 CA repeats sequence is presented in table 2. It must be mentioned that genotypes with 19 repeats of CA dinucleotide or more are depicted as L and the ones with smaller are considered as S. Among 10 different genotypes presented in table 2, five ones were absent in control individuals including three SL genotypes (17\19, 17\20 and 17\21) and two LL (19\22 and 20\22) ones. When SS genotype was compared to LL homozygotes, an RR value of 0.977 (p=٠,٦٢٧), was observed (Table 3). Furthermore, individuals with SL genotype tend to increase RR value up to 1.7 folds when compared to each of SS and LL genotypes (p=0.06٦ and p=0.03١ respectively). Comparing SL heterozygous with both SS and LL genotypes, a significant increase of RR value up to 4.5 folds (p=0.03٠) was recorded as illustrated in table 3.

Table 2. Genotype distribution of IGF1 CA repeats sequence

Genotype	Number of CA repeats in allele1\allele2	Number of genotypes (%)	
		Patients	Control individuals
SS	18\18	10 (7.35)	11 (8.08)
SS total		10 (7.35)	11 (8.08)
SL	17\19	1 (0.75)	0 (0.00)
	17\20	2 (1.51)	0 (0.00)
	17\21	2 (1.51)	0 (0.00)
	18\20	4 (3.03)	2 (1.47)
SL total		9 (6.62)	2 (1.47)
LL	19\19	104 (76.47)	110 (80.88)
	19\21	4 (3.03)	3 (2.2)
	19\22	1 (0.75)	0 (0.00)
	20\20	7 (5.30)	10 (7.35)
	20\22	1 (0.75)	0 (0.00)
LL total		117 (86.03)	123 (90.44)
Total		136 (100)	136 (100)

Table 3. IGF1 Genotype association with prostate cancer development

IGF1 genotypes	Patients	Control	RR (95% CI)	p value
SS \ LL	117\10	123\11	0.977 (0.642-1.633)	0.627
SL\LL	9\117	2\123	1.678 (1.234-2.282)	0.031
SL\SS	9\10	2\11	1.718 (1.013-2.913)	0.066
SL\SS and LL	9\127	2\134	4.500 (0.990-20.444)	0.030

Age adjusted, family history, BPH and prostate cancer risk

An age cutoff of 70 was used for further genotypic analysis as depicted in table 4. After the age adjustment, an RR value elevation was observed when we compared the SS with LL individuals, but it was not significant (p=0.627). Comparing SS and LL patients based on their family history of prostate cancer, showed a small insignificant elevation of RR value (RR=1.248, p=0.519). Conversely, comparing SS patients with LL ones albeit based on their BPH history, a significant (p=0.016) RR value of 3.143 was recorded (Table 4).

Table 4. IGF1 Genotype association with prostate cancer development

Patients genotypes	SS	LL	RR (95% CI)	p value
age adjusted*	6\1	60\32	1.314 (0.543-1.066)	0.254
family history**	2\5	19\64	1.248 (0.363-4.296)	0.519
BPH history***	4\0	14\30	3.143 (2.039-4.844)	0.016

*Age adjusted at 70 years old, therefore men with age equal or larger than 70 were compared with those smaller than 70 years old.
**Men with family history of prostate cancer were compared with men without that family history
***Men with BPH history were compared with men without that history

Discussion

Overexpression of IGF1 in transgenic mice declared the emergence of adenocarcinoma along with 50% tumors formation (10). Taking into account the 21 eligible studies in human subjects show that high concentrations of IGF-I could increase (odds ratio or OR=1.49, 95% CI: 1.14-1.95) the risk of prostate cancer development (11). As we know, the interactions between environmental and genetic factors can prime cancer incidence; moreover, the association of gene polymorphisms and the increased risk of prostate cancer have been proposed frequently (12-16). In our research, the polymorphism of IGF1 promoter region was studied in Isfahan province of Iran to clarify the possible association of this site with the incidence of prostate cancer.

As a pioneer study highlighting the IGF1 polymorphic site, homozygous Japanese men carrying $(CA)_{19}$ allele showed

an increased risk (OR= 3.36, p=0.012) of prostate cancer development (17). Afterward, a decreased risk (OR= 0.50; 95% CI: 0.27-0.93) of prostate cancer reported paradoxically for the same homozygotes but in western countries (18). By the same token, supporting results from black (OR=0.3, 95% CI: 0.1-0.7) and white (OR=0.4, 95% CI: 0.1-1.6) Americans confirms the protective role of the $(CA)_{19}$ allele (19). Unfortunately, other studies also in the USA, obfuscate any association between $(CA)_{19}$ allele and prostate cancer incidence (20, 21). Similarly, we also could not show any association between IGF1-$(CA)_{19}$ allele and prostate cancer incidence in our study population (Table 1). Unexpectedly, we found that $(CA)_{17}$ super-small allele could significantly (p=0.006) increase the risk of prostate cancer development up to two folds (Table 1). Our super-large $(CA)_{22}$ allele was also able to increase the risk of developing prostate cancer, but insignificantly (OR= 2, p=0.083. Table1). Comparing of LL or SS genotypes with SL heterozygotes showed 1.7 folds increased risk of prostate cancer among hterozygots (Table 3). Increased RR values of 4.5 folds were recorded significantly based on combine analysis of SL individuals with both of LL and SS ones (Table 3). In accordance, Japanese $(CA)_{19}$ heterozygotes showed an increased risk of prostate cancer development (OR= 1.78, p=0.001). Therefore, heterozygote advantage or over-dominance could explain our results since heterozygotes, but not homozygotes, increased the RR value (22). According to our results, none of the age and family history could significantly associate with the development of prostate cancer (Table 4). In line with our results, Tsuchiya et al also could not show any significant association between $(CA)_{19}$ allele, the tumor stage and grade in Japanese patients (17). On the other hand, they concluded that IGF-I $(CA)_{19}$ heterozygote and homozygote could significantly (p=0.009, p=0.012 respectively) increase the risk of BPH in Japanese population (17). We also showed that SS genotypes could significantly increase (p=0.002) the risk of BPH development more than 3 times before cancer incidence (Table 4). In conclusion, carriers of $(CA)_{17}$ allele could be at a higher risk of prostate cancer development and SL heterozygotes are at a higher risk of BPH development in Isfahan province of Iran.

Conflict of interest

None of the authors has any conflicts of interest to disclose and all authors support the submission to this journal.

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